

New England Bioterrorism Preparedness Workshop

T.J. Dasey

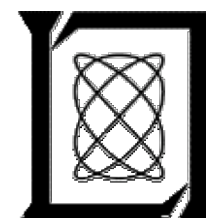
H.M. Sapolsky

3-4 April 2002

Lincoln Laboratory

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Military Biological Weapons Programs

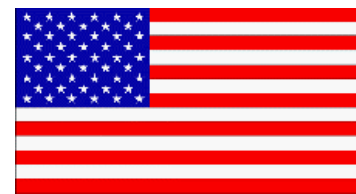
Geoffrey Forden

Security Studies Program, MIT

April 3, 2002

We now know what terrorists want from Biological Weapons, but what does a military want?

I will examine two programs we know a lot about:



Voluntarily eliminated BW program in 1969 and declassified much information



Forced to “eliminate” program and much information gained from inspectors. 1

Estimates of Historical Weapons Effectiveness

Combatants



- Precision Guided Munitions: 0.75 Casualties/ton
- World War I Chemical Weapons: 10 Casualties/ton (0.2 deaths/ton)
- Iran-Iraq War Chemical Weapons: ~35 Casualties/ton

Civilians



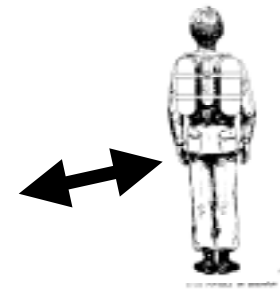
- 1995 Tokyo Sarin Attack (CW): ~2200 Deaths/ton
- Atomic Bomb (Hiroshima): ~100,000 Deaths/ton
- Anthrax Attacks (October Incident): ~1,000,000 Deaths/ton
- Thermonuclear: ?

The U.S. Biological Weapons Program

1. Artillery shells



2. Stationary generator left behind by special forces.



3. Boat mounted line spray source



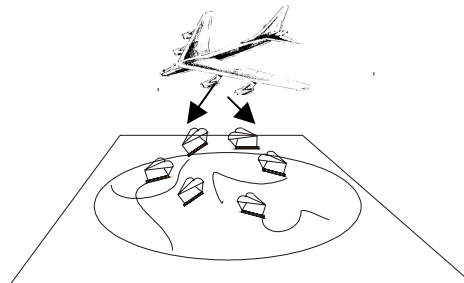
4. Naval point source



5. Self-dispersing spheres



6. Flettner rotors



7. Drone mounted line spray source.

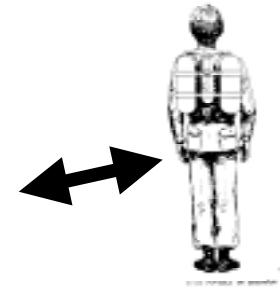


1. ~~Artillery shells~~

~~Abandoned~~



2. Stationary generator left behind by special forces.

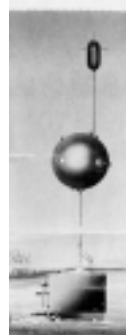


3. Boat mounted line spray source



4. ~~Naval point source~~

~~Abandoned~~



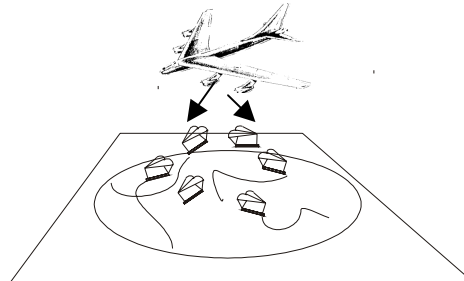
5. Self-dispersing spheres

~~Obsolete~~



6. ~~Flettner rotors~~

~~Obsolete~~



7. Drone mounted line spray source.



Overview of Various BW weapon Systems.



Military Uses of Biological
Weapons:
Serious Suggestions, Far from
being actual war plans!

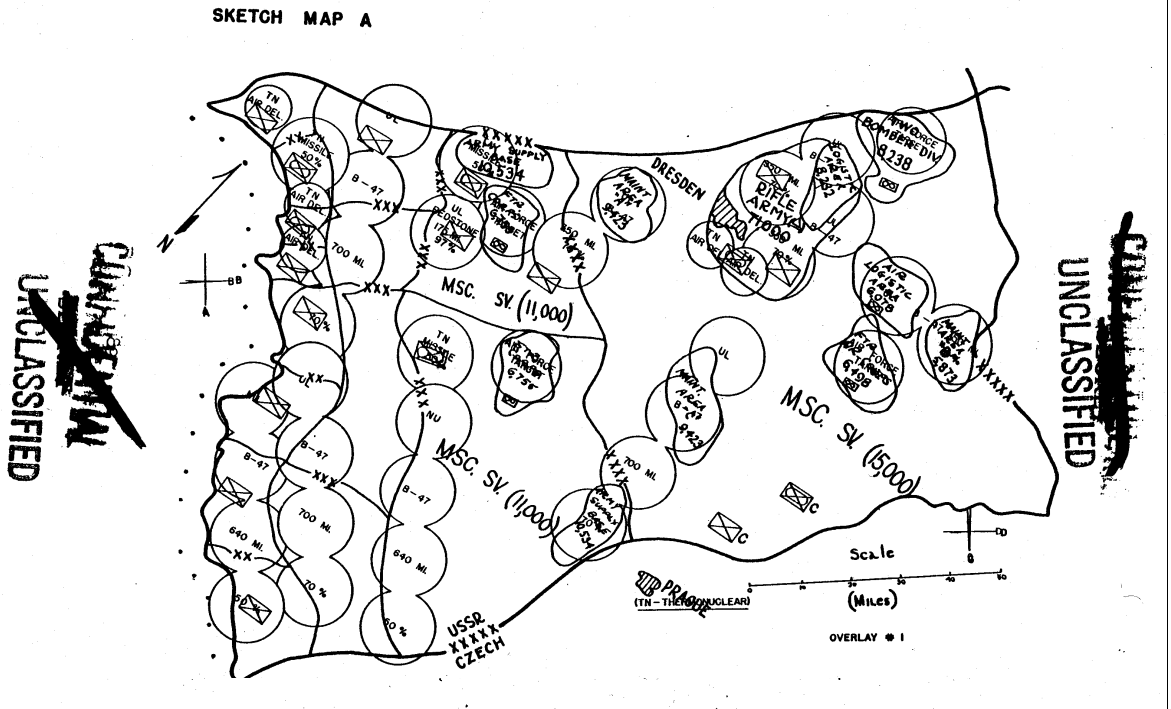
A map of Europe and the surrounding North Atlantic region. The map includes latitude lines at 30°N, 40°N, 50°N, and 60°N, and longitude lines at 10°W, 0°, and 30°E. A red hatched rectangle is drawn in the North Atlantic, indicating the location of the study area, which is situated west of the British Isles and north of the Azores.



UNCLASSIFIED

Using Tactical BW in General War: Blunting a Soviet Attack (ca. 1958)

Resources required
for BW mission:



| | Military | Civilian |
|------------------|----------|----------|
| AW Killed | 31,800 | 12,000 |
| AW Injured | 31,800 | 12,000 |
| AW Total | 63,600 | 24,000 |
| BW Killed | 39,600 | 46,000 |
| BW Incapacitated | 118,800 | 134,500 |
| BW Total | 158,400 | 180,500 |
| GRAND TOTAL | 222,000 | 204,500 |



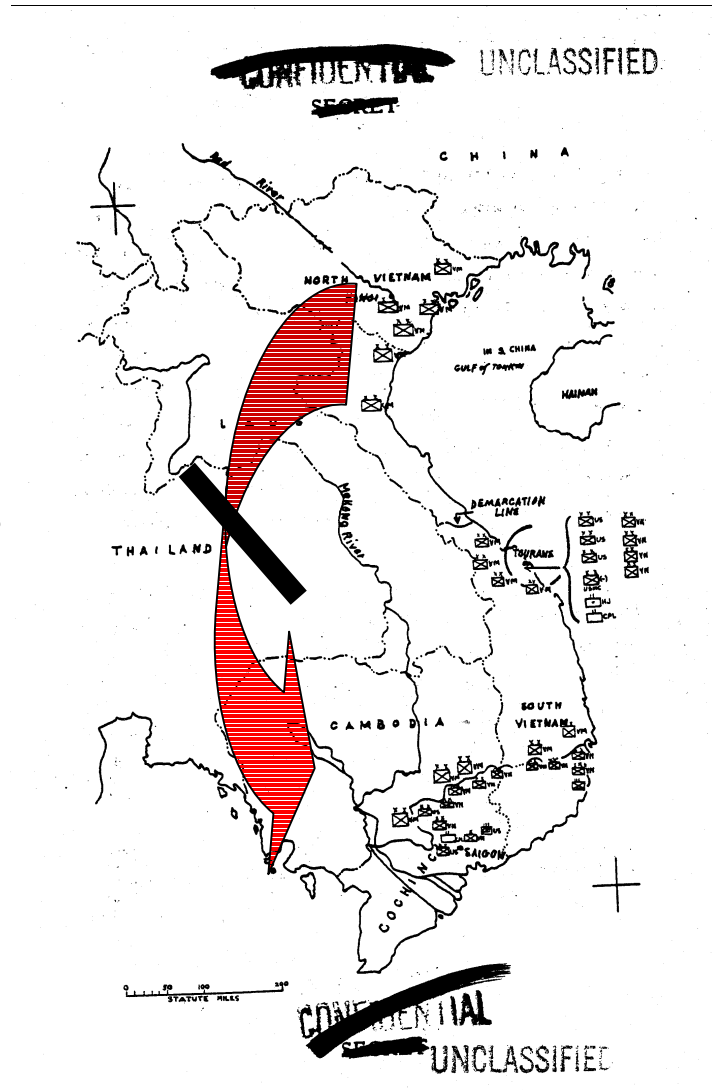
+thermonuclear weapons in the north

NOTE: Total ground troops in southern army front, 429,250.

Using “Strategic” BW in a Peripheral War: blunting a Soviet Attack (ca. 1958)

Goals:

- 1) Block troop transfer from the North to the South.
- 2) Incapacitate enemy troops in the South to reduce their effectiveness at defense.



Assumption:

US fights a static defense until all its forces are in place. It can then launch an offensive at a time of its choosing, i.e. 7 days after BW attack.

Resources required for BW mission:

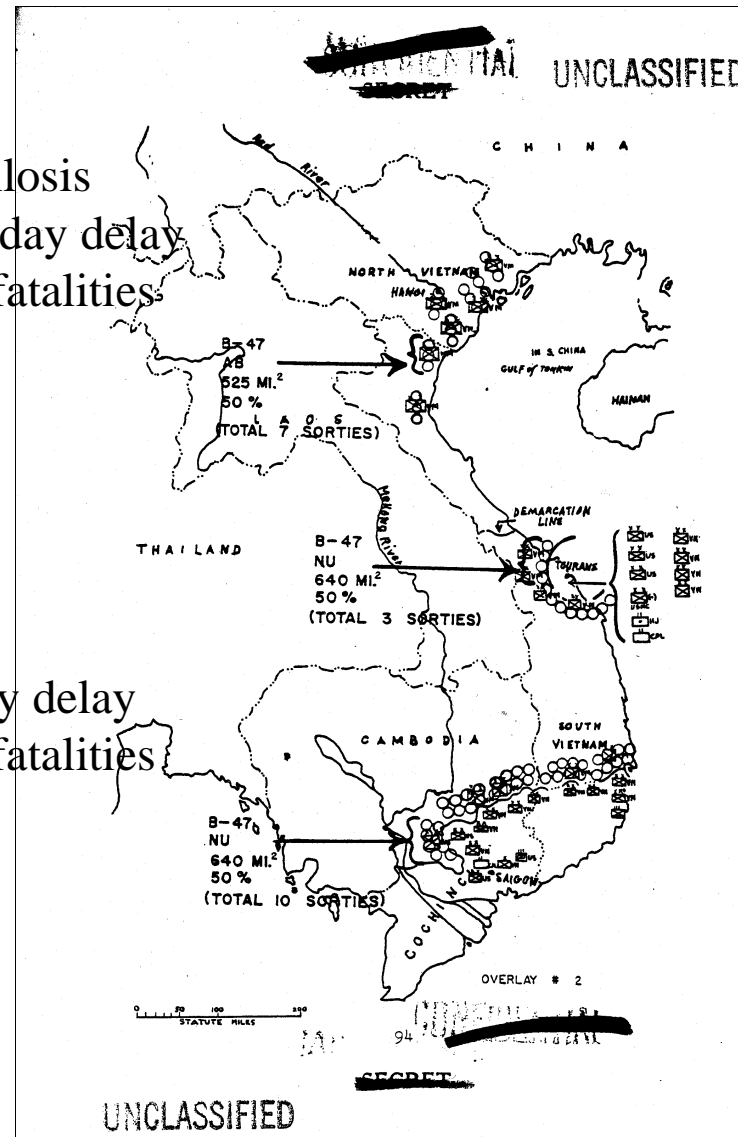


| <u>Saigon and Tourane Perimeters</u> | | | |
|--------------------------------------|-----------------|-----------------|--|
| | <u>Military</u> | <u>Civilian</u> | |
| Total in Area | 137,000 | 243,200 | |
| Total Incapacitated | 68,500 | 130,000 | |
| Total Lethalities | 0 | 0 | |
| Total Casualties | 68,500 | 130,000 | |
| <u>Tonkin Delta</u> | | | |
| | <u>Military</u> | <u>Civilian</u> | |
| Total in Area | 84,000 | 1,128,000 | |
| Total Incapacitated | 40,200 | 497,000 | |
| Total Lethalities | 800 | 11,000 | |
| Total Casualties | 41,000 | 508,000 | |

UNCLASSIFIED

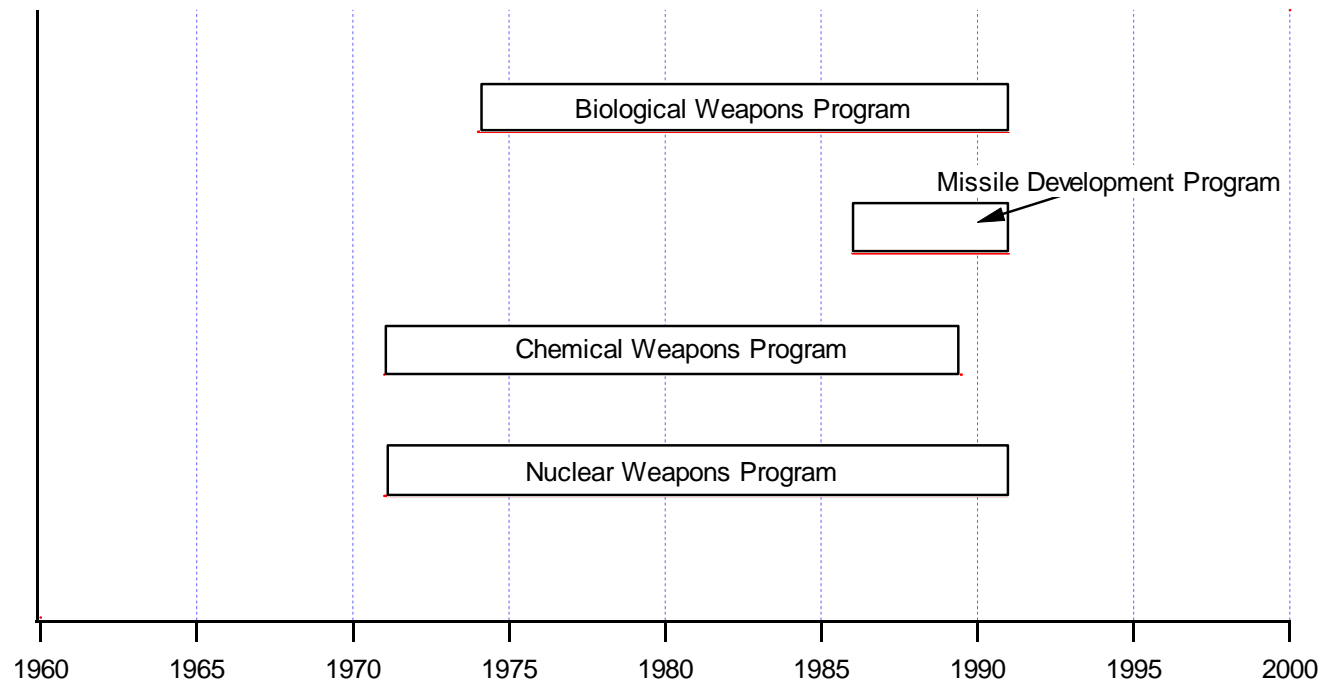
Brucellosis
12-15 day delay
< 2% fatalities

VEE
2-5 day delay
< 2% fatalities

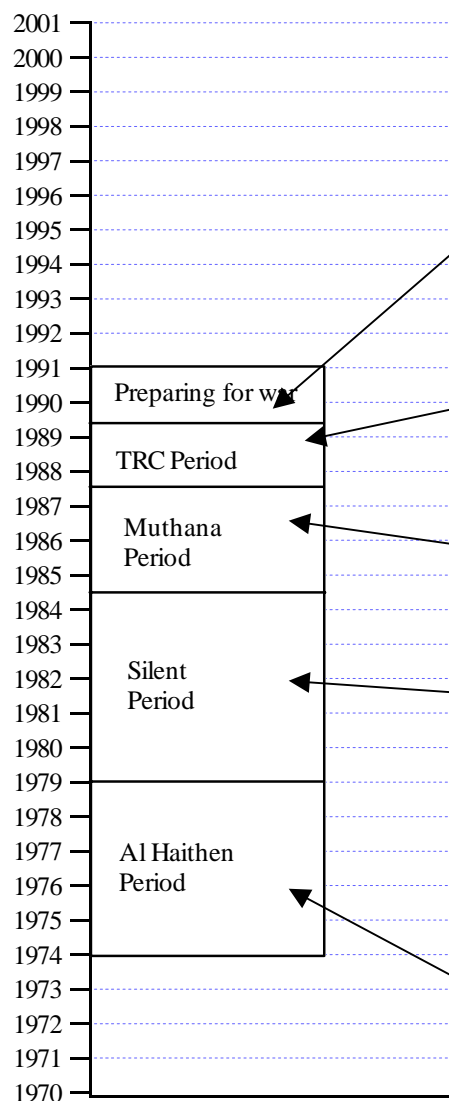


Iraq's Biological Weapons Program

Timeline of Iraq's WMD Programs



The Different Periods of Iraq's BW Program



Concepts of operations were formed but UNSCOM does not know much about these since they are prohibited from inquiring about military matters.

A procurement network was established (for importing needed items from outside the country) and planning took place, but not much else is known about Iraq's activities.

This period was dedicated to weapons development, with some research.

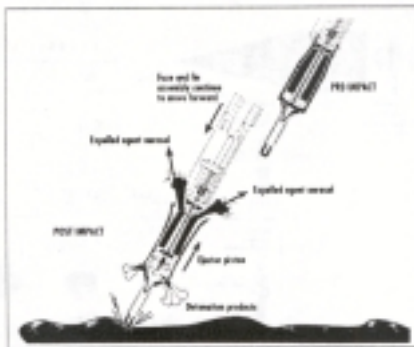
This was primarily a training period with students sent abroad for PhDs (mostly to the UK). Existing facility was closed. During this period, Iraq isolated bacteria and viruses, investigated drying processes, taught students, and built up an institute.

During this period, the goals of the program were “dirty tricks” associated with assassination of political opponents (staining shirts with ricin) and protection of the President (food poison testing). But the program failed with people being sent to jail because of fraud, for instance. However, the people who ended up leading the BW program were associated with it at this period.

Weaponization of Iraq's BW Agents

Biological Munitions

Example—modifying a Cardoen PM-1 submunition



Iraq tried to set up a factory for these tactical warheads that could be filled with either CW or BW. They are made to look just like normal HE 155 mm rounds, presumably to prevent Iranians from taking CBW defense measures.

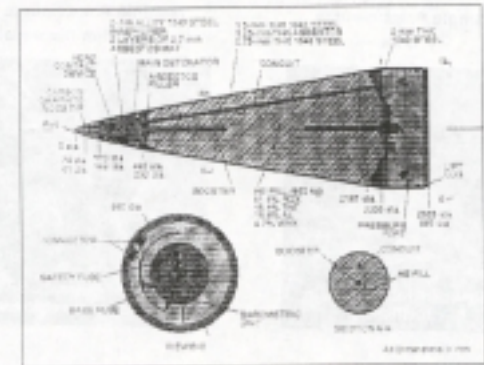
UAV delivery of BW



Iraq tried to develop remotely controlled Mirage fighters with large drop tanks filled with BW, even into the Gulf War.

Bio Warhead—Munitions

Bulk fill high explosive theater missile



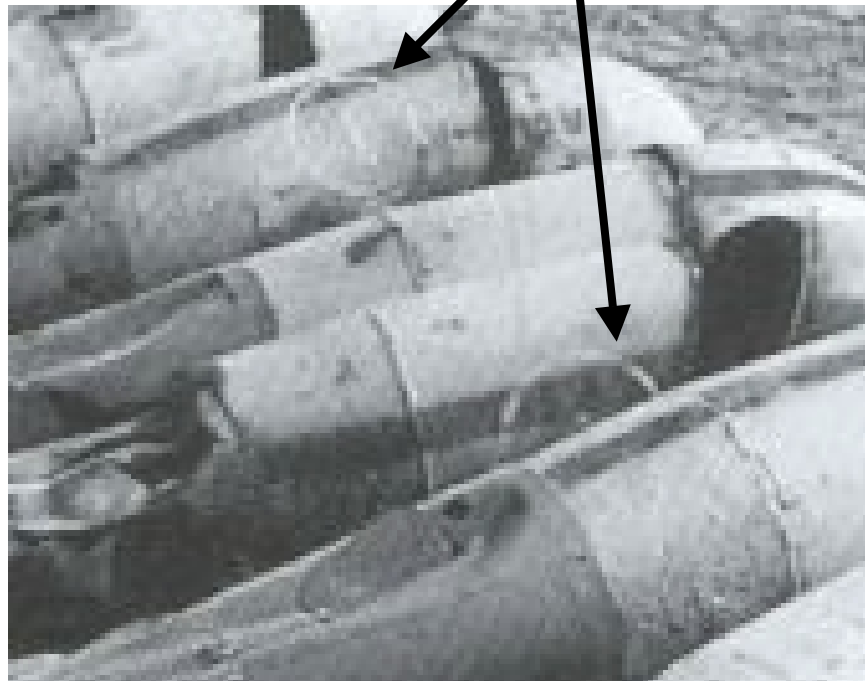
25 SCUD warheads were filled with anthrax. These were filled in the period before the 1991 Gulf War.

Weaponization of Iraq's BW Agents (Cont')



These gravity bombs were filled with CW, as indicated by the empty circle. BW bombs had an Arabic A in side the circle.

CW markings,
BW bombs had an Arabic
“A”



Supergun—A new way of getting Intercontinental Distances



A supergun (termed little Babylon), with 350 mm diameter, as actually built but apparently not tested. This version was built as a fixed direction/launch angle. But it could have been weaponized to a more mobile version.

FIGURE 57 PAYLOAD CAPABILITIES OF HIGH ALTITUDE PHOENIX

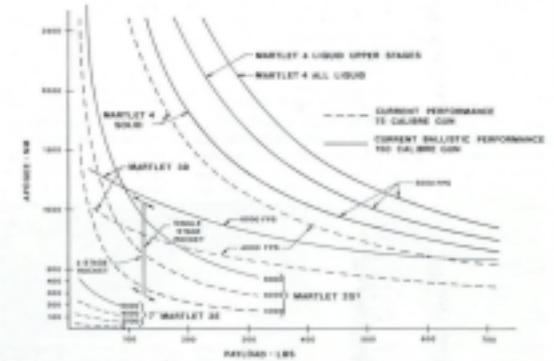
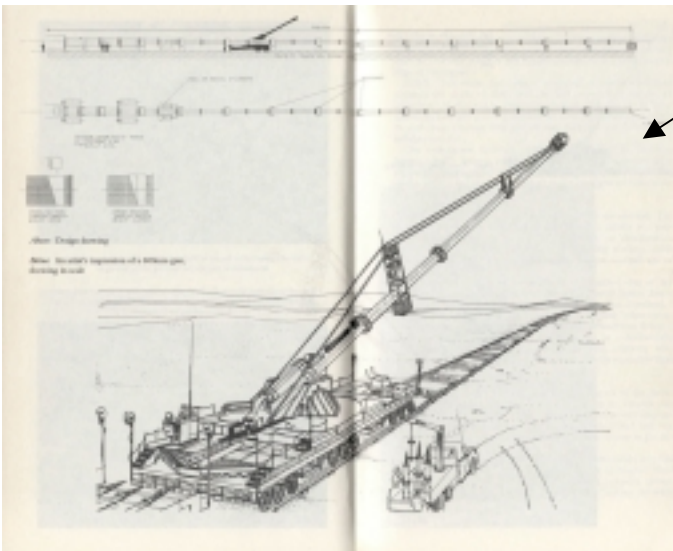
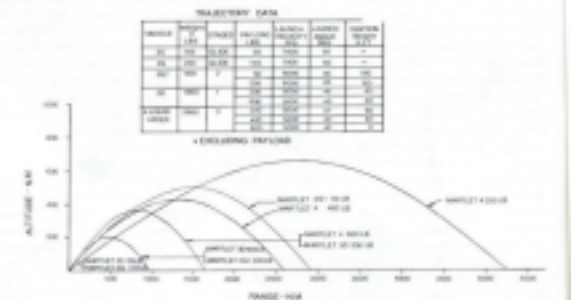


FIGURE 58 LONG RANGE PERFORMANCE, 16 INCH GUN



Clandestine BW Dispersal



Anthrax and Smallpox: Comparison of Two Outbreaks

Jeanne Guillemin

Senior Fellow

MIT Security Studies Program

Anthrax and Smallpox: Comparison of Two Outbreaks

- 1979 Sverdlovsk anthrax epidemic, officially explained by consumption of infected meat; military aerosol suspected
- 1972 Yugoslavia smallpox epidemic, started by a pilgrim returning from Mecca via Baghdad, site of unreported outbreak

Key Problem = Late Diagnosis

1. What are the political causes?
2. What are the medical/professional causes?
3. What are the public communication causes?

1979 Sverdlovsk Epidemic

1992-1994 investigation of an
“unnatural” outbreak of inhalational
anthrax

Sources of Evidence

- KGB list of 64 victims' names and addresses
- Interviews with families/neighbors of 56 victims
- Cemetery data
- Autopsy tissue data
- Hospital records (5 survivors)
- Local hospital and factory clinic lists
- Veterinary documents/animal deaths

16-I, 19-I, в мае было выявлено 20 больных (рис.3).



Распределение больных по датам с учетом непродолжительности инкубационного периода, позволило исключить инфицирование через мясо, поступавшее на питание населения в централизованном порядке. Ясно, что в этом случае следовало ожидать взрывообразное нарастание заболеваемости. Растянутый характер вспышки приняла из-за длительного хранения мяса населением. Так, в конце апреля в семье Г., состоящей из 2-х человек пенсионеров, о наличии мяса, купленного в начале месяца, удалось узнать только в итоге продолжительной беседы. Мясо в данном случае не вызывало опасений, поскольку уже несколько раз добавлялось при варке студня. В семье учительницы С. часть жирного мяса была перетоплена для получения сала, которое использовалось в пищу. Однако из мяса, изъятых в указанных семьях, было выделено два штамма возбудителя сибирской язвы. Информация населения, передававшаяся через местное радио и печать об опасности употребления случайно купленного мяса, создала уверенность в том, что мясо все изъято и уничтожено, не сохранилось у населения, это поэтому настоятельные меры для его выявления и изъятия не принимались. Вместе с тем, наблюдения в ряде очагов показали также возможность инфицирования

Page from Soviet report,
1988, submitted to
US State Department.

April 4-May 16, 1979 cases
reported as due to eating
infected meat over weeks.
Fatalities 64, survivors 15.

Anna Komina
Ceramics factory
worker, age 54;
resident of
affected district

Date of onset of
symptoms: April 4
Date of death: April 10





Valentin Petrovich Borisov
Age 27, Soldier, Compound 32



Pyotr Pilyasov, Age 39
Construction worker



June, 1992, Hospital 20, in Ekaterinburg's southern Chkalovsky district. Team members Martin Hugh Jones, veterinarian, Alexis Shelokov, virologist, and Matthew Meselson, biochemist and team organizer, with a university host V. A. Shpetkin, and the hospital director, Margarita Ilyenko.



Street leading towards ceramics factory (smokestack in Center) where 18 workers died of anthrax, April-May 1979



1993. Interior of pipe shop of abandoned ceramics factory. Large, third-story windows on left face northwest.



Gate of Compound 19 military base, southwest Ekaterinburg. Soldier is allowing truck to enter.



Cottage in village southeast of Ekaterinburg where animals died of anthrax in 1979, starting April 5-6, and where villagers were vaccinated and quarantined.

Sverdlovsk, c.1985

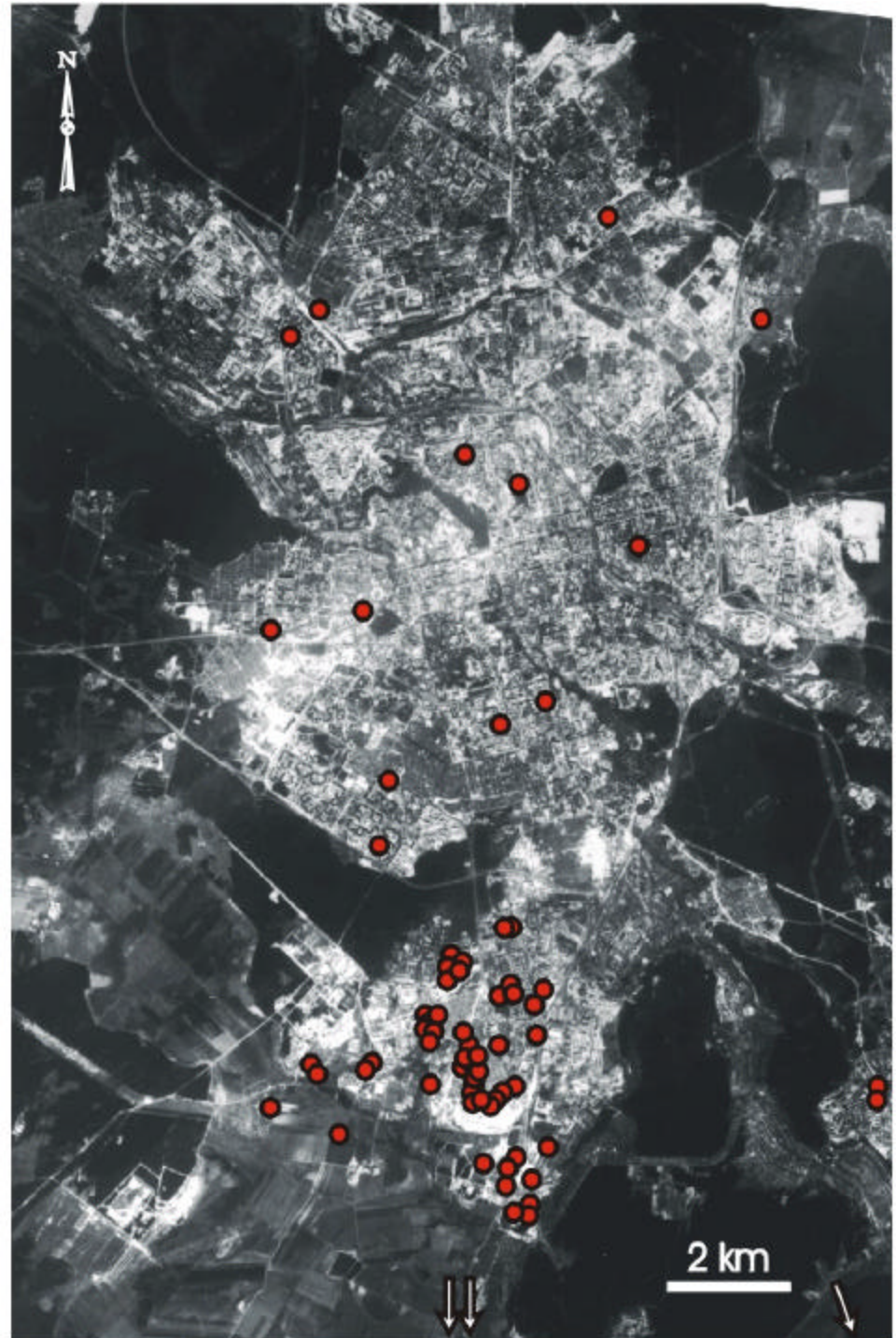
Red dots=Nighttime

Locations of victims.

Addresses obtained from
KGB and other lists.

Southern cluster is in
Chkalovsky rayon.

Arrows=homes off map.



Chkalovsky District Only (note inset of entire city)

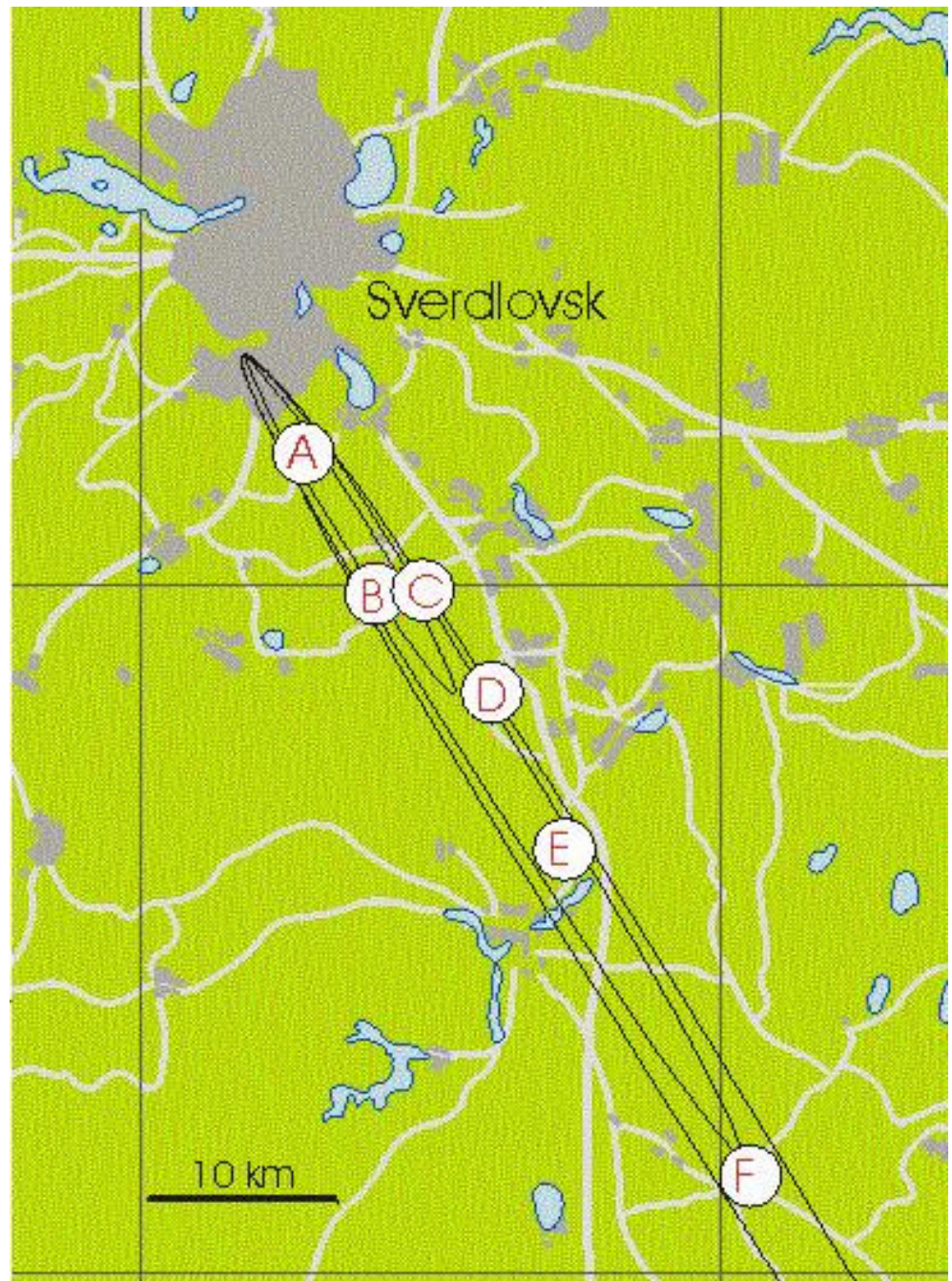
Irregular white lines show
Compounds 19 and 32.

White rectangle indicates
Ceramics factory.

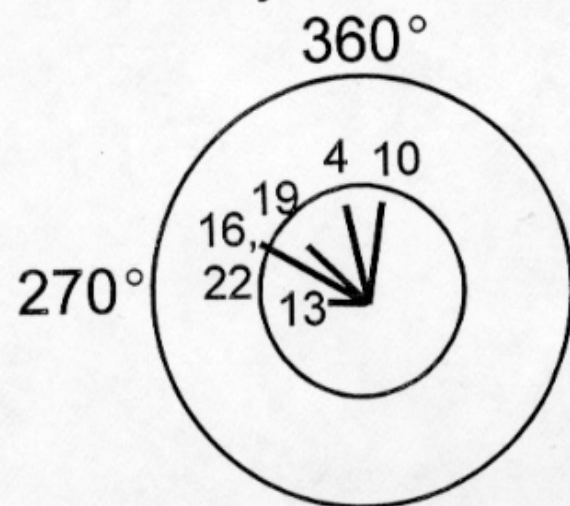
Red dots=daytime locations of
66 victims and 11 survivors.



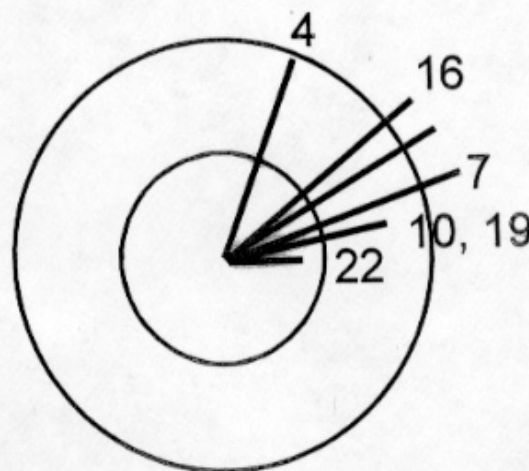
Six villages southeast of
Sverdlovsk where
1979 epizootic occurred.
Public health measures
April through May.
Interviews conducted at
F, Abramovo, confirmed
Veterinary documents.



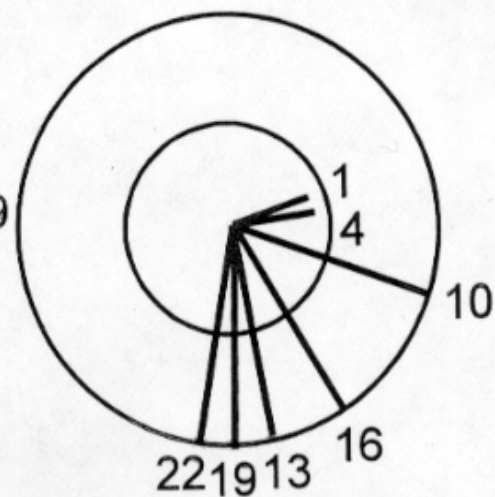
Friday March 30



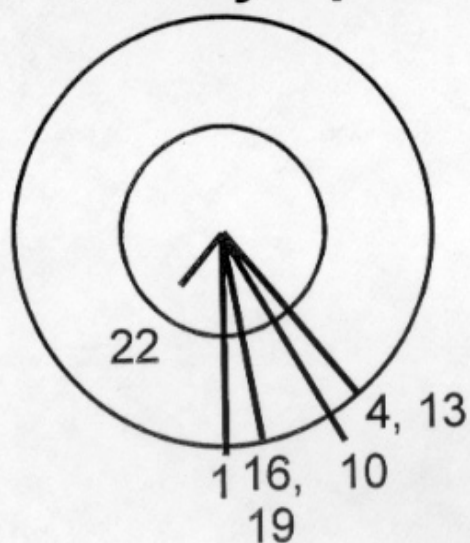
Saturday March 31



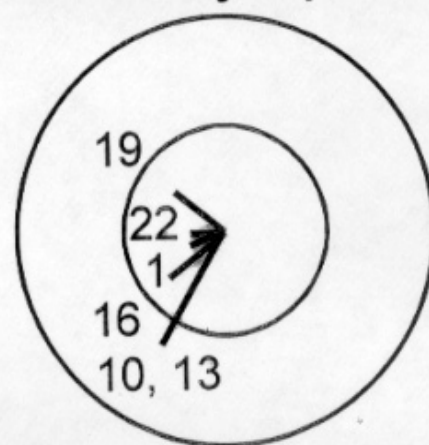
Sunday April 1



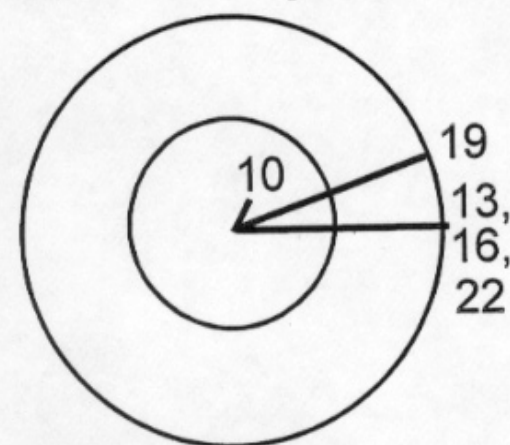
Monday April 2



Tuesday April 3



Wednesday April 4

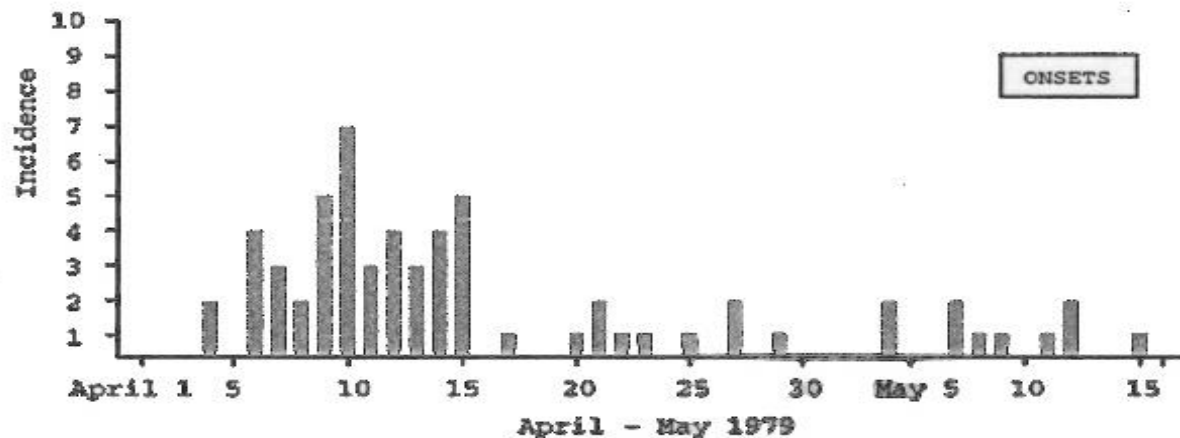


Research Findings

- A lethal emission of anthrax spores from Compound 19 occurred during the afternoon of April 2, 1979.
- No young people under 24 or children were affected.
- Approximately 80 people (of some 5000 exposed) became infected; 11 survived with treatment.
- An estimated gram (a trillion spores) caused the fatalities; attack rate of 1-2%; fatality rate around 80% (note late diagnosis).
- Inhalation anthrax in humans can occur as long as 43 days after exposure. (First evidence in human cases)

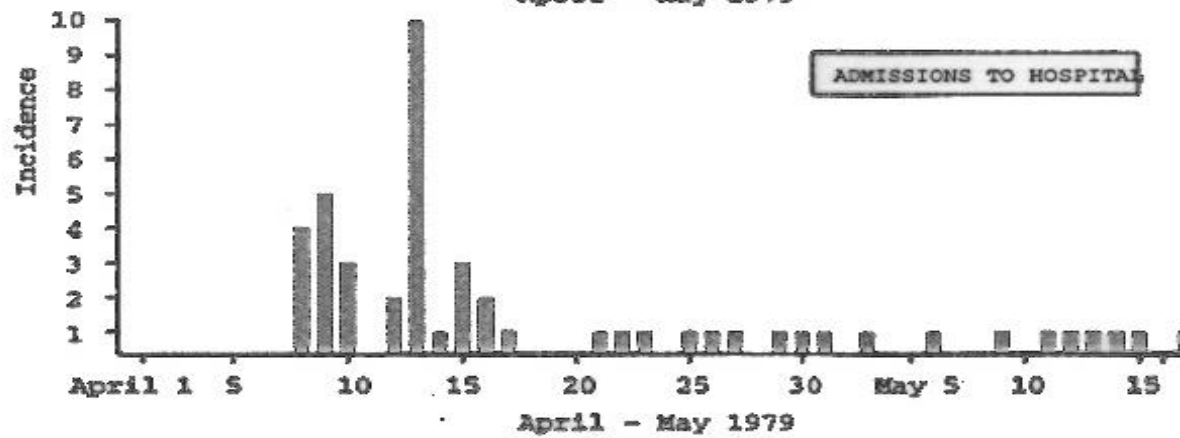
Soviet Public Health Response

- Urban: lab diagnosis, screening for central hospital intensive care and pediatric cases, ambulance transport, autopsy team; 4000 volunteers mobilized for disinfection and distribution of antibiotics; Moscow clinical team, vaccine campaign for 50,000; building exteriors washed.
- Rural: roadblocks, carcasses burnt, enforced human vaccination, animal sheds destroyed, 3-week village quarantine.



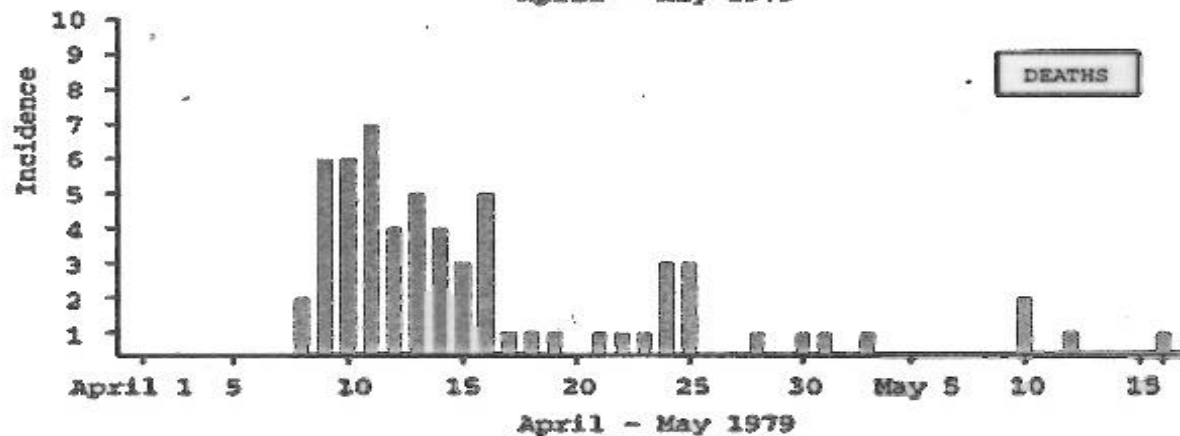
Diagnosis 9 days post
April 2 exposure
Total 21 deaths

Moscow doctors
April 12 arrival.
Total 25 deaths



17 victims die with no
hospital care

City clean-up begun.
30,000 vaccinated.
April 16,
Total 42 deaths



Last recorded death
May 16.
Total 66 valid cases
11 survivors

Smallpox Epidemic Yugoslavia, 1972

Imported Virus Contagion
“Natural Outbreak”

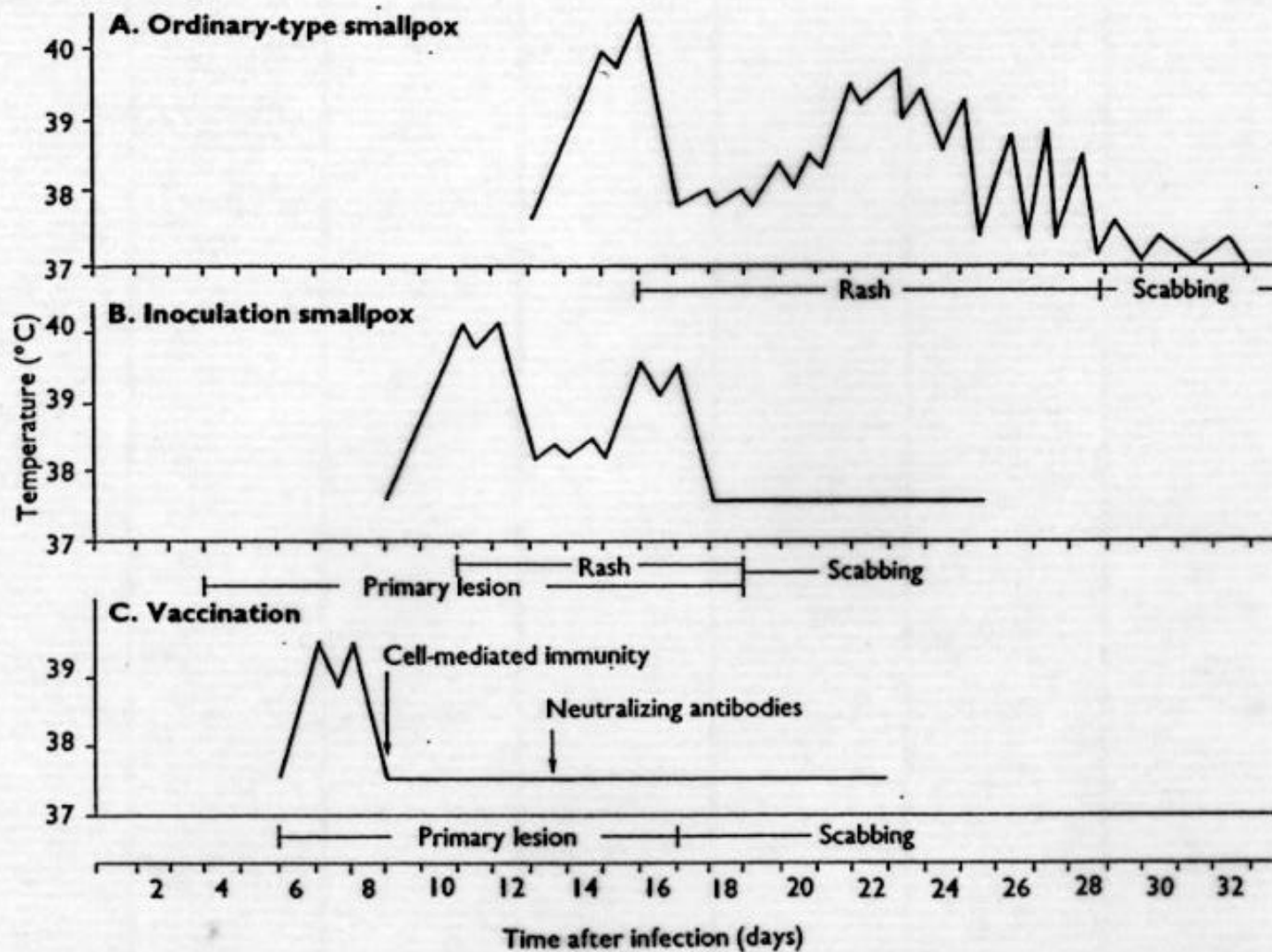


Fig. 1.3. The clinical course of moderately severe ordinary-type variola major in an unvaccinated subject (A); inoculation smallpox (variola) in an unvaccinated subject (B); and primary vaccination (C). (Temperature records from an illustration in Hime (1896) with modified wording.)

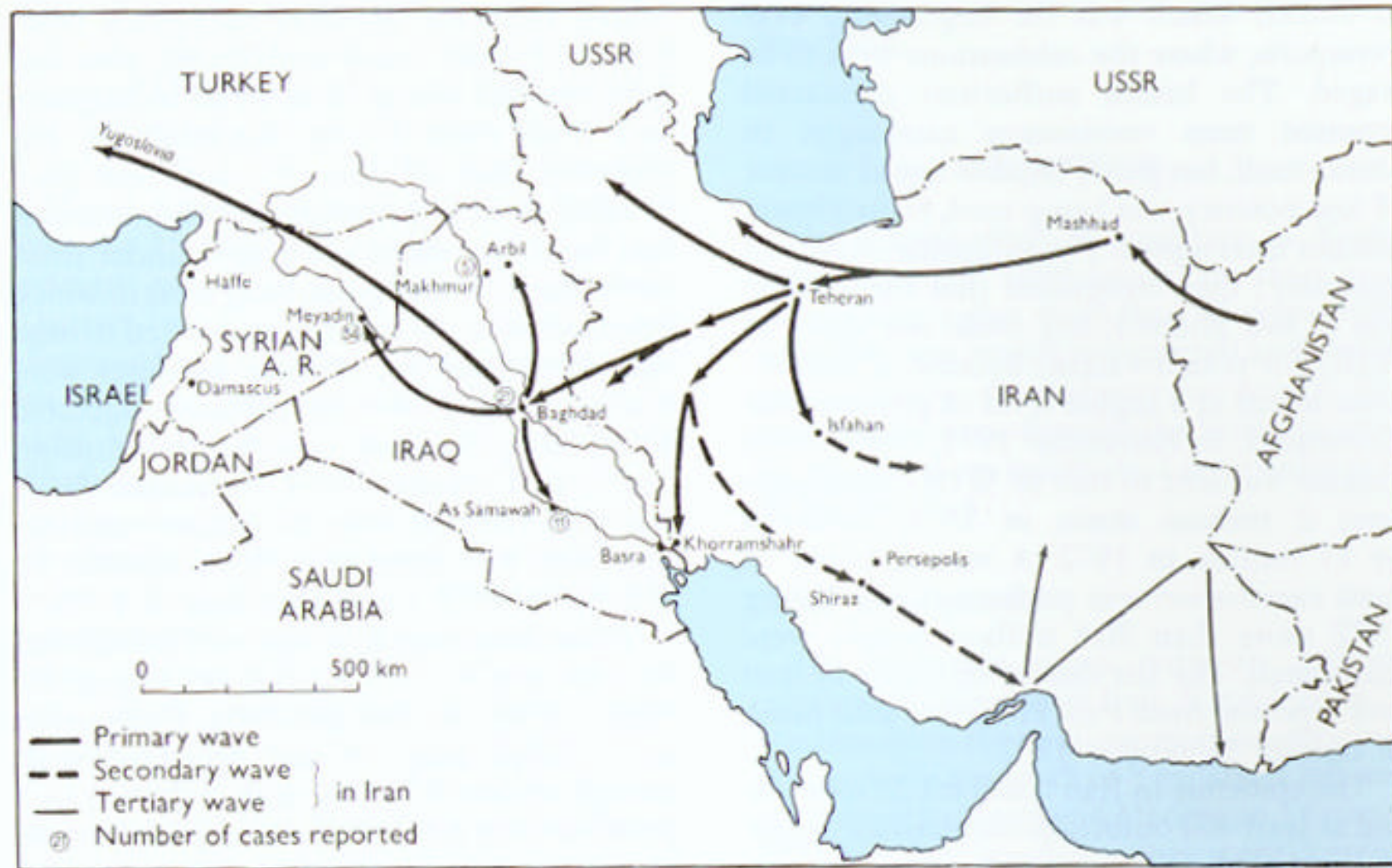
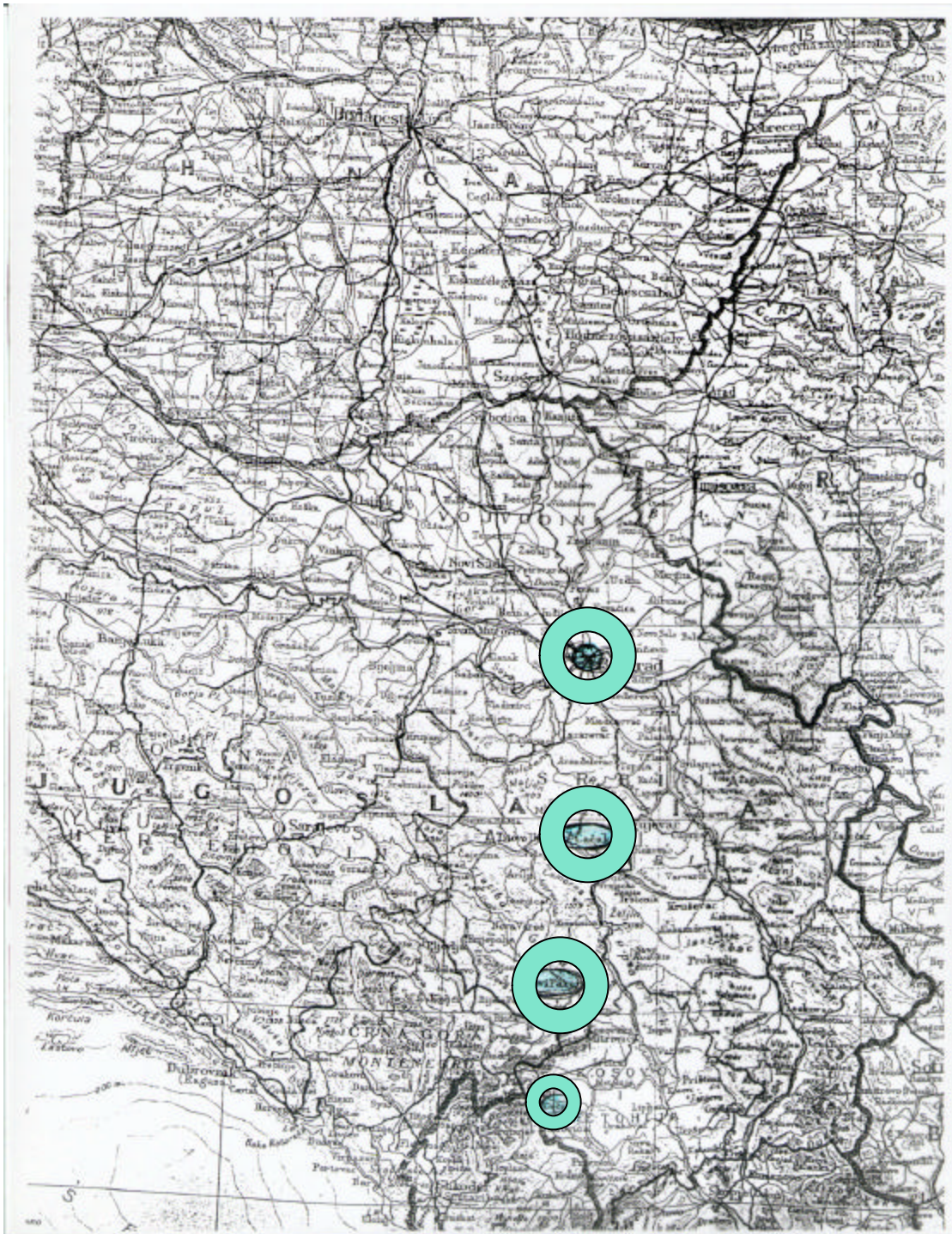


Fig. 23.5. Spread of smallpox in Iran, Iraq and the Syrian Arab Republic, 1970–1972. The disease was introduced from Afghanistan into Mashhad, Iran, in October 1970. There were three waves of dispersion through Iran, which lasted over a period of 22 months. By the end of 1971 smallpox had crossed into Iraq, where it spread north to Arbil and south to As Samawah. Transmission in Iraq was interrupted by June 1972. In February 1972, smallpox spread from Baghdad in Iraq to Meyadin in the Syrian Arab Republic, where a smaller outbreak occurred that was contained by June 1972.



Feb. 3-7 index case infected in Baghdad.

Feb.15-16 falls ill at home Danjani (Kosovo)

Mar.5 one of 11 infected by index case falls ill in Serbia

Mar.10 Serbian dies after infecting 42 in hospital

Mar.11, Serbia case total 10, Kosovo 12

Mar.13 physician in Kosovo sounds alert

Mar.17 diagnosis and state containment initiative

Mar.25 case total is 137

April 15 case total is 173
(123 Kosovo, 48 Serbia, 1 Vojvodina, 1 Montenegro)

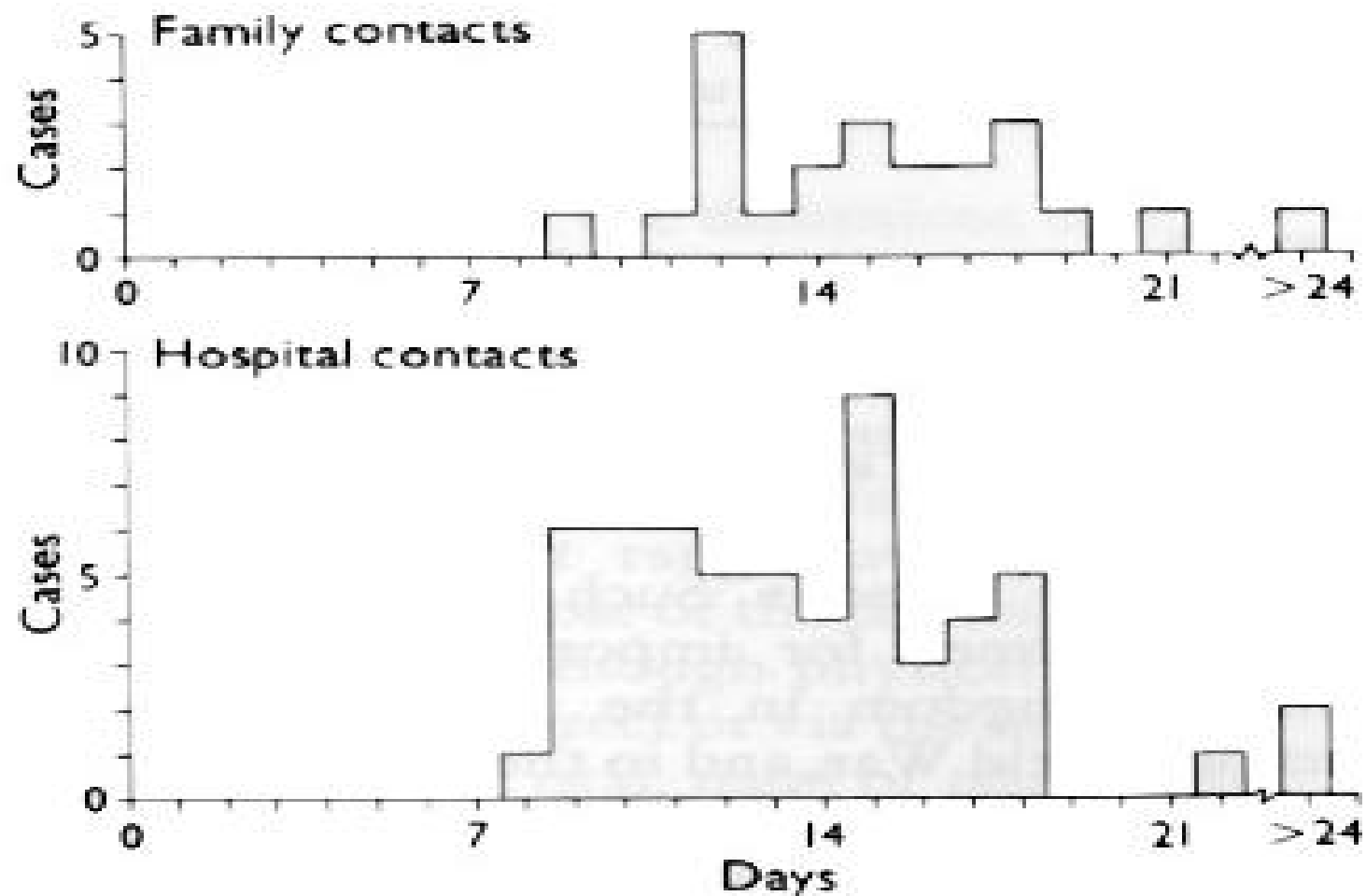
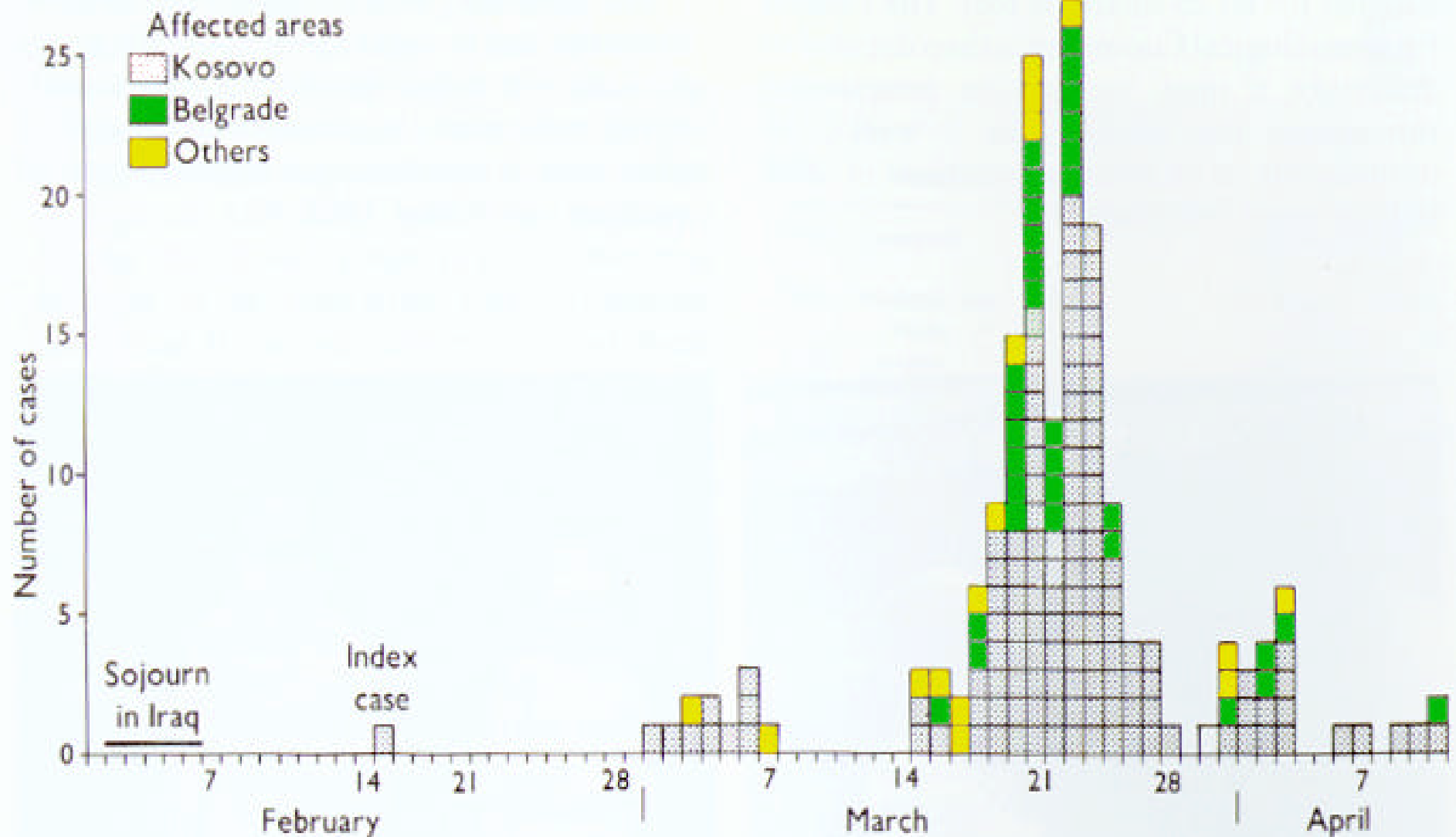


Fig. 4.8. The interval between the first possible exposure to a case of smallpox imported into Europe by air and the onset of symptoms in first generation indigenous cases, in family and hospital environments. (Based on Mack, 1972.)





Public Health Response
 Mar. 15 to May 9
 Vaccine campaign,
 Quarantine, roadblocks.
 Belgrade team joins
 Kosavar local health staff
 (rural, many migrant
 workers) to begin
 concentric circles of
 Vaccinations in 25 foci,
 with family and village
 quarantine, prohibition
 of public meetings.
 18 million (of 20.8
 million citizens) were
 vaccinated in 3 weeks.
 175 cases, 35 dead (20%)
 case fatality rate. 37% of
 cases among previously
 vaccinated.

Structural Sources of Late Diagnosis

- Political: military secrecy/religious repression
- Medical/Professional: lack of familiarity with disease (misdiagnosis)
- Communication: public uneducated about risk

Solutions to Late Diagnosis

1. Political-public health cooperation
2. Medical technology and education
3. Accurate public communication

US Preparedness for Biological Terrorism

Gregory Koblentz
Security Studies Program
MIT

April 3, 2002

Overview

- Background and History
- September 11 and Anthrax Letters
- Preparedness Post-September 11

Background and History

- 1996 Nunn-Lugar-Domenici Domestic Preparedness Program
- 1998 White House Initiative
 - Pharmaceutical Stockpile
 - Grants to State Public Health Agencies
 - Metropolitan Medical Response System
 - Research & Development

Figure 1. HHS Spending on Bioterrorism Preparedness and Research, 1998-2002

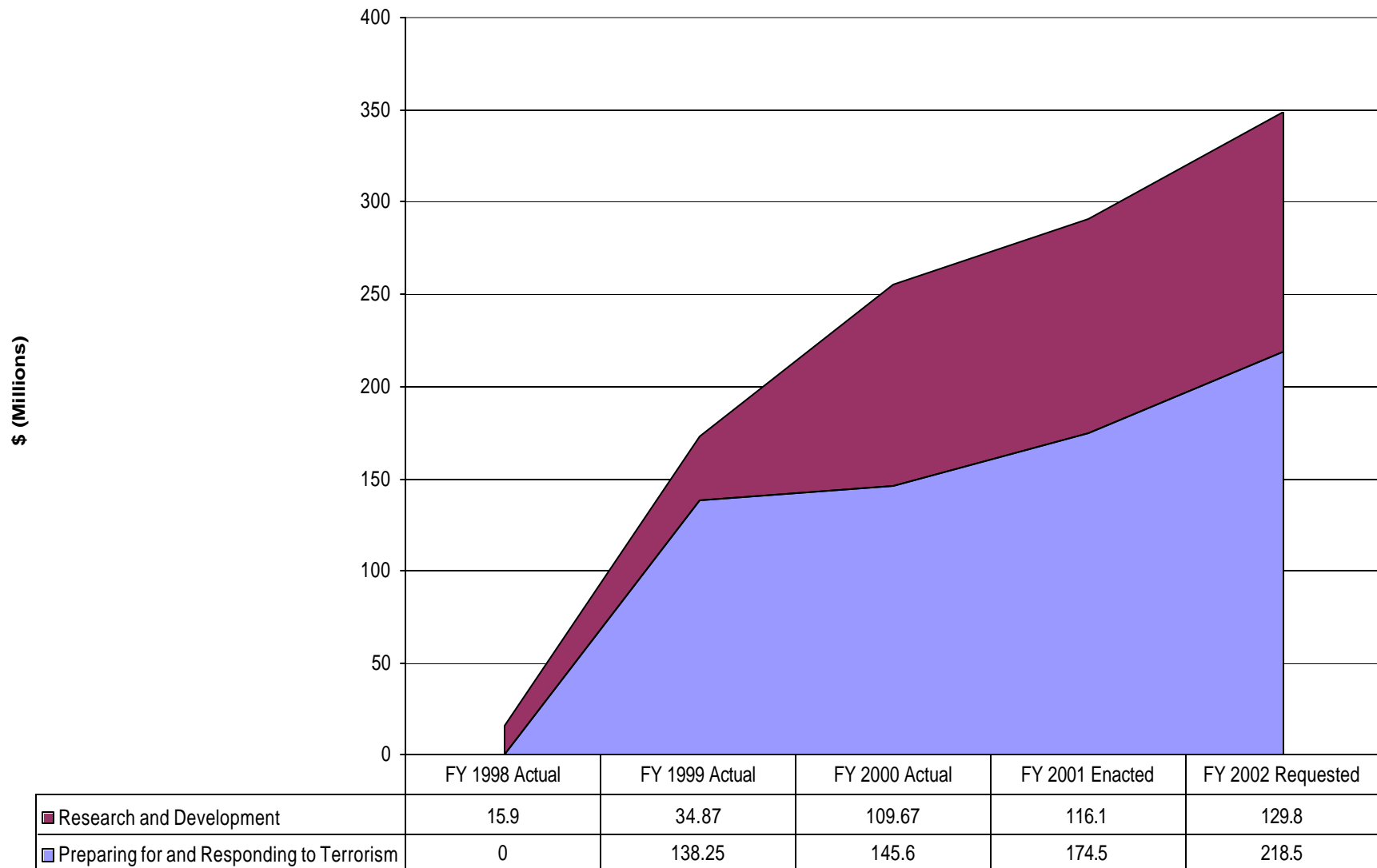
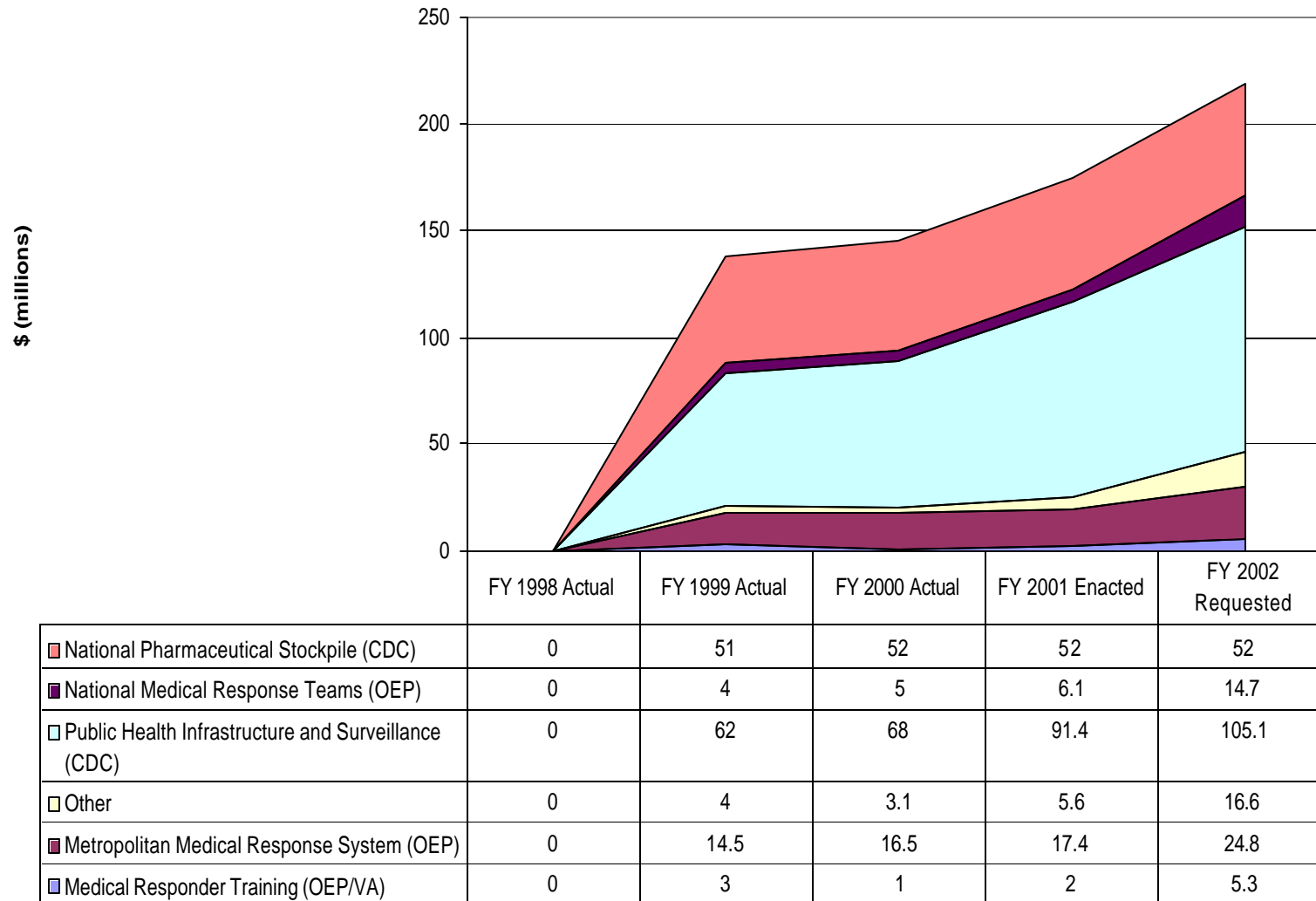


Figure 2. HHS Spending on Bioterrorism Preparedness, 1998-2002

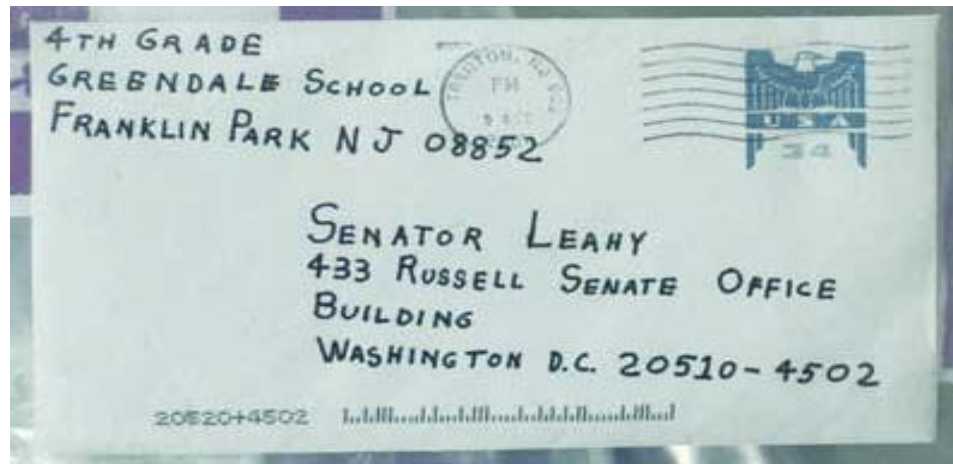


Bioterrorism Preparedness Prior to September 11

- State and Local Laboratory, Surveillance and Epidemiological Capabilities Improving Slowly
- Pharmaceutical Stockpile in Place
- Hospitals and Healthcare Providers Neglected

The Anthrax Letters

- 5 letters each with ~2 grams of anthrax
- 23 confirmed cases: 5 fatalities
- > 10,000 on antibiotic prophylaxis
- Cost of response: \$250 million



Lessons Learned

1. Expect the Unexpected
2. Doctors are the First Line of Detection
3. Early Treatment is Key
4. Lab Capacity Needs to Be More Robust
5. Coordination and Communication Problems
6. Flawed Knowledge Assessment
7. Importance of Forensics

Figure 3. HHS Spending on Bioterrorism Preparedness and Research, 1998-2003

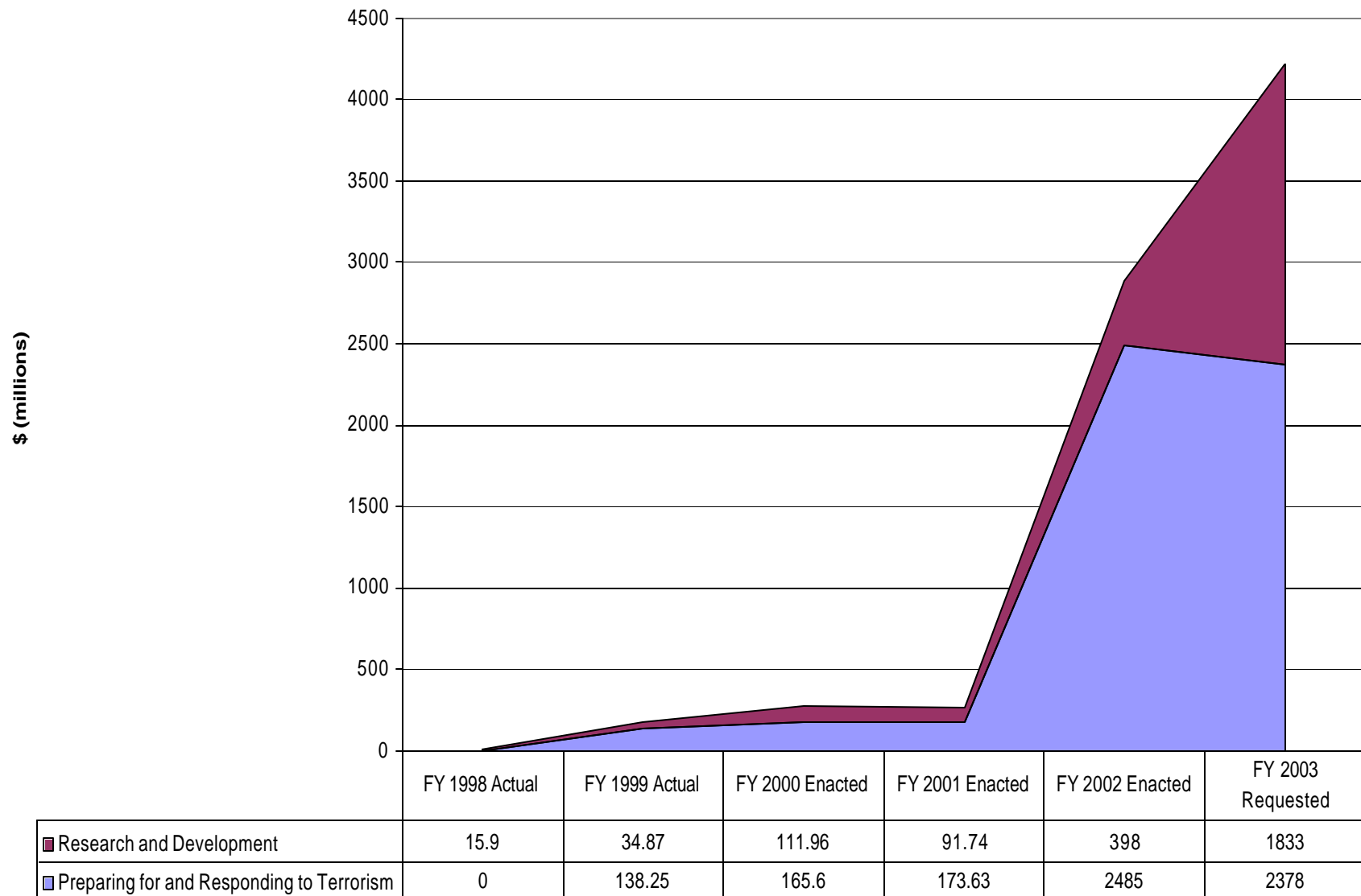
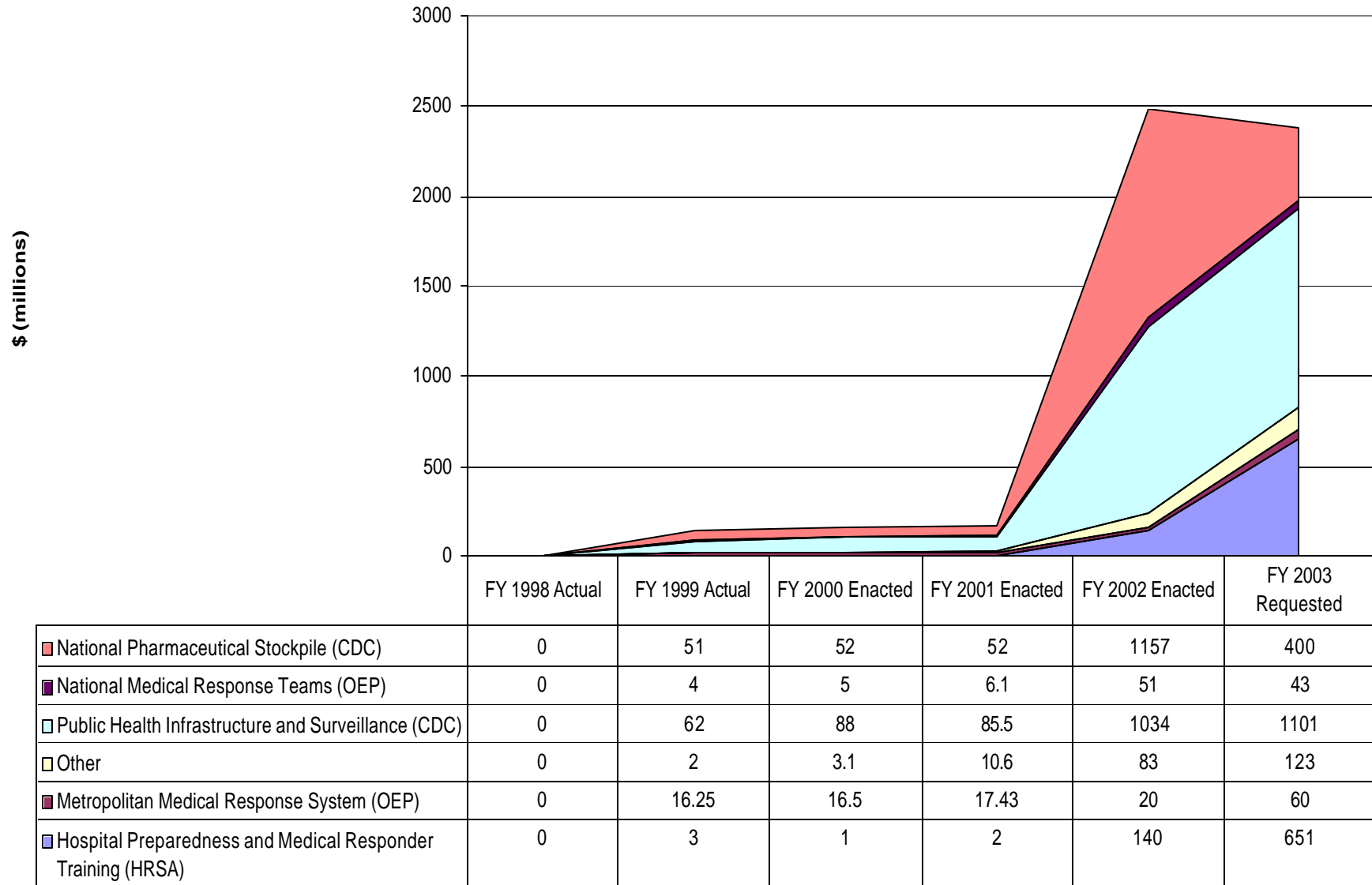


Figure 4. HHS Spending on Bioterrorism Preparedness, 1998-2003



Post-September 11 Preparedness

- Existing Programs Expanded and/or Accelerated
 - Public Health Infrastructure
 - Pharmaceutical Stockpile
 - Metropolitan Medical Response System
 - Research and Development
- New Programs Established
 - Hospital Preparedness
 - Medical Responder Training

Assessment of Post-September 11 Preparedness

- National Strategy is Required
 - Interagency
 - Intergovernmental
 - Interdisciplinary
 - Public-Private
- Bioterrorism is NOT Another Emerging Infectious Disease
 - Mother Nature vs. Bin Laden
 - Criminal and National Security Implications

Public Health Surveillance: A local health department perspective



M. Anita Barry, MD, MPH
Director, Communicable Disease Control
Boston Public Health Commission

Objectives

- Current public health surveillance
- Characteristics of the ideal surveillance system
- Boston's enhanced surveillance system for bioterrorism and mass casualty events
- Future plans

Types of Surveillance

- Notifiable disease reporting
- Active surveillance
- Laboratory based surveillance
- Population based surveillance

Notifiable Disease Reporting

- Health care providers are required by law or regulation to notify public health about:
 - Named pathogens
 - Specified diagnoses
 - Outbreaks or clusters of illness
- Usually a passive system, but can use enhanced passive technique
- Reporting requirements differ among states

Notifiable Disease Reporting: Why it's incomplete

- Unaware of the requirement to report
- Confused about the mechanics of reporting
- Concern about confidentiality
- Someone else's job
- Unconfirmed case (wrong diagnosis, no lab)
- Forgot to do it

Active surveillance

- Public health staff review records and other data on site (for example, at a hospital)
- Provides fairly complete data
- Very labor intensive and requires a sustained effort - resources become a problem

Laboratory based surveillance

- Laboratories are required to report certain positive test results to public health
- Isolated laboratory data are incomplete
 - False positives, false negatives
 - Skewed testing (publicity, specific signs and symptoms)
- Molecular microbiologic techniques enhance epidemiologic investigations

Population Based Surveillance

- Illness in closed communities (such as incarcerated populations)
- Absenteeism rates
- Insurance claims data
- Sales of specific products (such as anti-diarrheal medications)

The Ideal Surveillance System

Fast, cheap, and easy...

The Problem

- Traditional surveillance systems based on the reporting of specific diseases have limited potential for early detection of mass casualty events such as bioterrorism or pandemic influenza.

Milwaukee: Cryptosporidium Infection Related to the Public Water Supply

- Estimated 400,000 people had outbreak associated diarrhea.
- 285 laboratory confirmed cases.
- Recognition of the outbreak was delayed:
 - Non-specific nature of the symptoms
 - Limited laboratory testing
 - Infrequent use of the health care system by people with diarrhea

Identification of the Outbreak

- Shortages of over the counter anti-diarrheal medications
 - pharmaceutical sales data impacted by sales & is unlikely to detect small case numbers
- Retrospective data indicated changes in health care utilization patterns prior to identification of the outbreak

Agents of Concern: CDC Category A

- *Bacillus anthracis* (anthrax)
- *Clostridium botulinum* toxin (botulism)
- *Yersinia pestis* (plague)
- variola major (smallpox)
- *Francisella tularensis* (tularemia)
- Viral hemorrhagic fever

Agents of Concern: CDC Category B

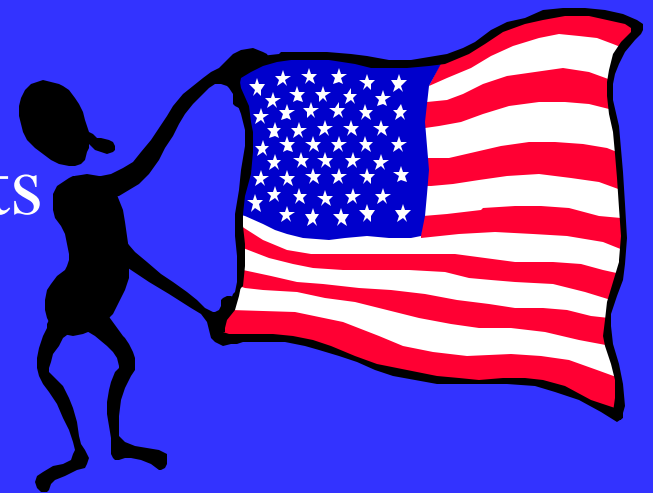
- *Coxiella burnetti* (Q fever)
- *Brucella* species (brucellosis)
- *Burkholderia mallei* (glanders)
- ricin toxin from *Ricinus communis* (castor beans)
- epsilon toxin of *Clostridium perfringens*

Agents of Concern: CDC Category C

- Nipah virus
- hantaviruses
- tickborne hemorrhagic fever viruses
- yellow fever
- multidrug-resistant tuberculosis

Bioterrorism Events in the United States

- 1984, The Dalles, Oregon
 - Salmonella in salad bars
 - 751 ill (45 hospitalized)
- 1996, Dallas, Texas
 - Shigella in micro-lab donuts
 - 12 ill (4 hospitalized)



Anthrax Cases, 2001

| Anthrax Among Outbreak-related Cases 2001 | | | | | | |
|--|----|-----|----|----|----|-------|
| Cases | FL | NYC | NJ | DC | CT | Total |
| Inhalational | 2 | 1 | 2 | 5 | 1 | 11 |
| Cutaneous | | | | | | |
| Confirmed | 0 | 4 | 3 | 0 | 0 | 7 |
| Suspected | 0 | 3 | 1 | 0 | 0 | 4 |
| Total | 2 | 8 | 7 | 5 | 1 | 22 |

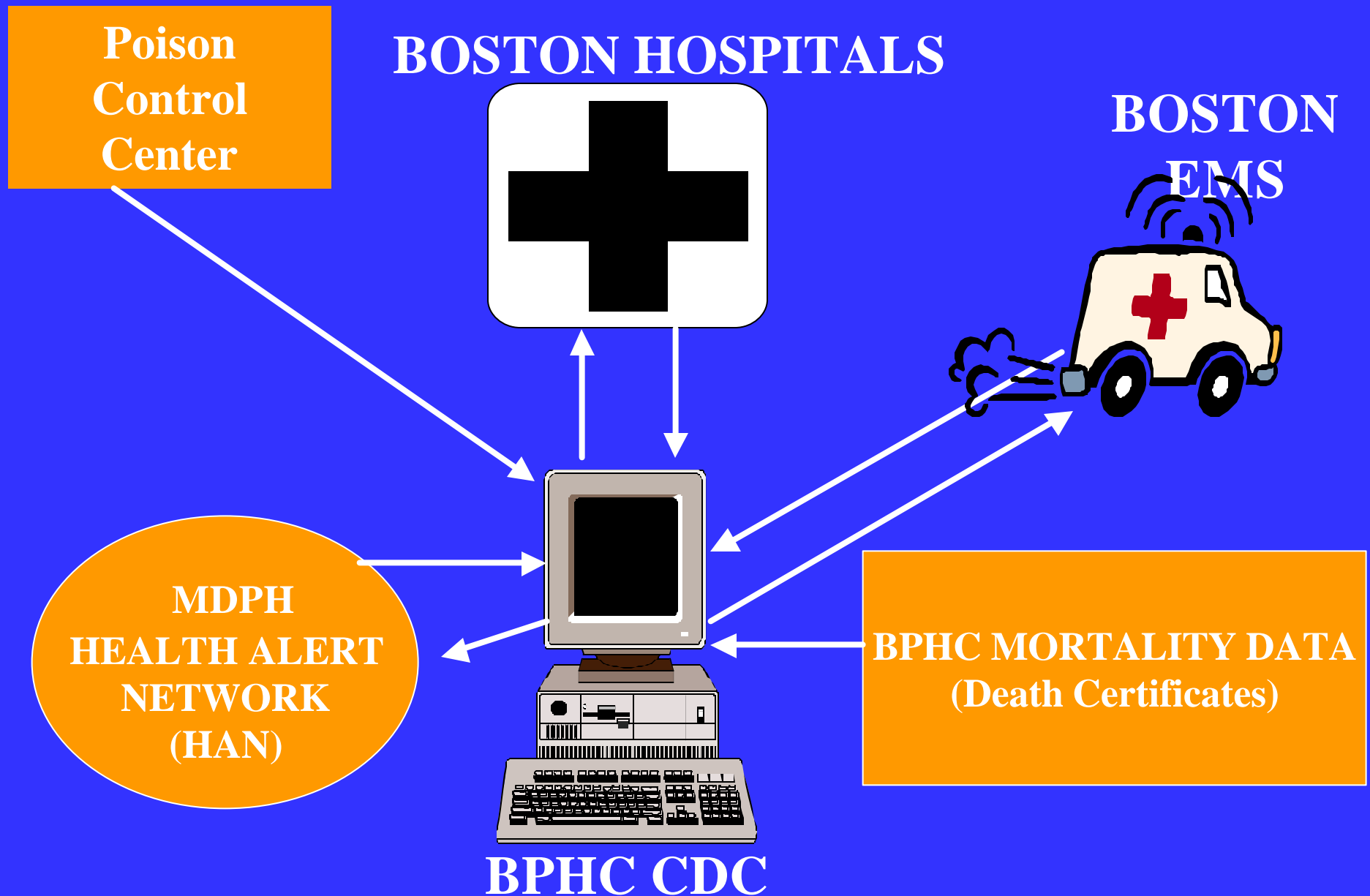
The Ideal Surveillance System

- Sensitive (with enough specificity to make it workable)
- Timely
- Provides complete data
- Cost effective
- Linked to an effective follow-up system to interpret initial signals

Enhanced Surveillance in Boston

- Emergency department visits
- Urgent care visits
- Boston EMS calls
- Death certificates
- Poison Control Center

VOLUME SURVEILLANCE SYSTEM DESIGN: DATA SOURCES



Enhanced Surveillance in Boston: Hospitals

- Every 24 hours volume data is electronically sent by SFTP to the Boston Public Health Commission (BPHC)
- Threshold data for each site based on historical data has been calculated
- If threshold is exceeded an initial assessment is automatically sent to an onsite contact

Calculations

Binomial distribution: adjust for month and day of the week

Number of events=average daily volume by month

n=Boston population (1990 census)

p= number of events/n

Upper CI= $p + ((1 - ?)(\sqrt{p(1-p)/(n)}))$

Upper threshold = Upper CI(n)

Enhanced Surveillance in Boston: Hospitals (Cont'd)

- If a cluster or any unusual cases of illness are identified on initial assessment, BPHC nurses/epidemiologists investigate further
- Data are typically available within 12 hours after the close of a 24 hour period

Enhanced Surveillance in Boston: Other Sites

- Poison Control Center: daily volume data being sent, thresholds being adjusted
- Boston EMS: type of calls of interest selected, automatic data transfer being developed
- Death Certificates: database developed; timeliness of data input being addressed

Enhanced Surveillance in Boston

Preliminary Findings

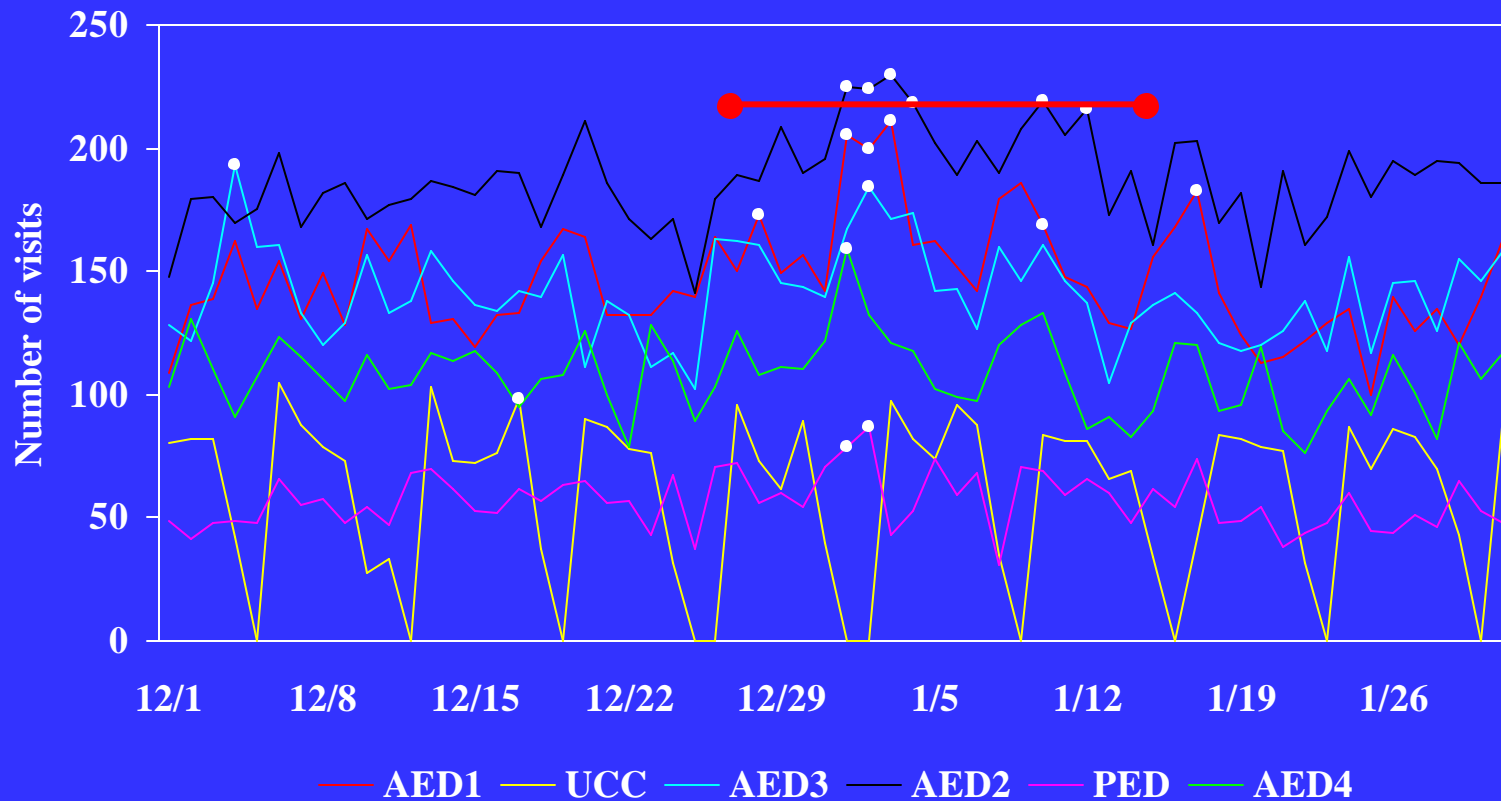
- System detected morbidity associated with a heat wave (retrospective)
- Volume data corresponded well with influenza activity in 1999 and 2000
- System identified changes in health seeking behavior post September 11

Volume data and influenza

- In 2000 there were 103 episodes of a site exceeding threshold.
- However, 3 or more sites simultaneously exceeded threshold on only 4 days and 2 sites on 17 days.
- Most of the time ($N=54$), only one site exceeded threshold on a given day.

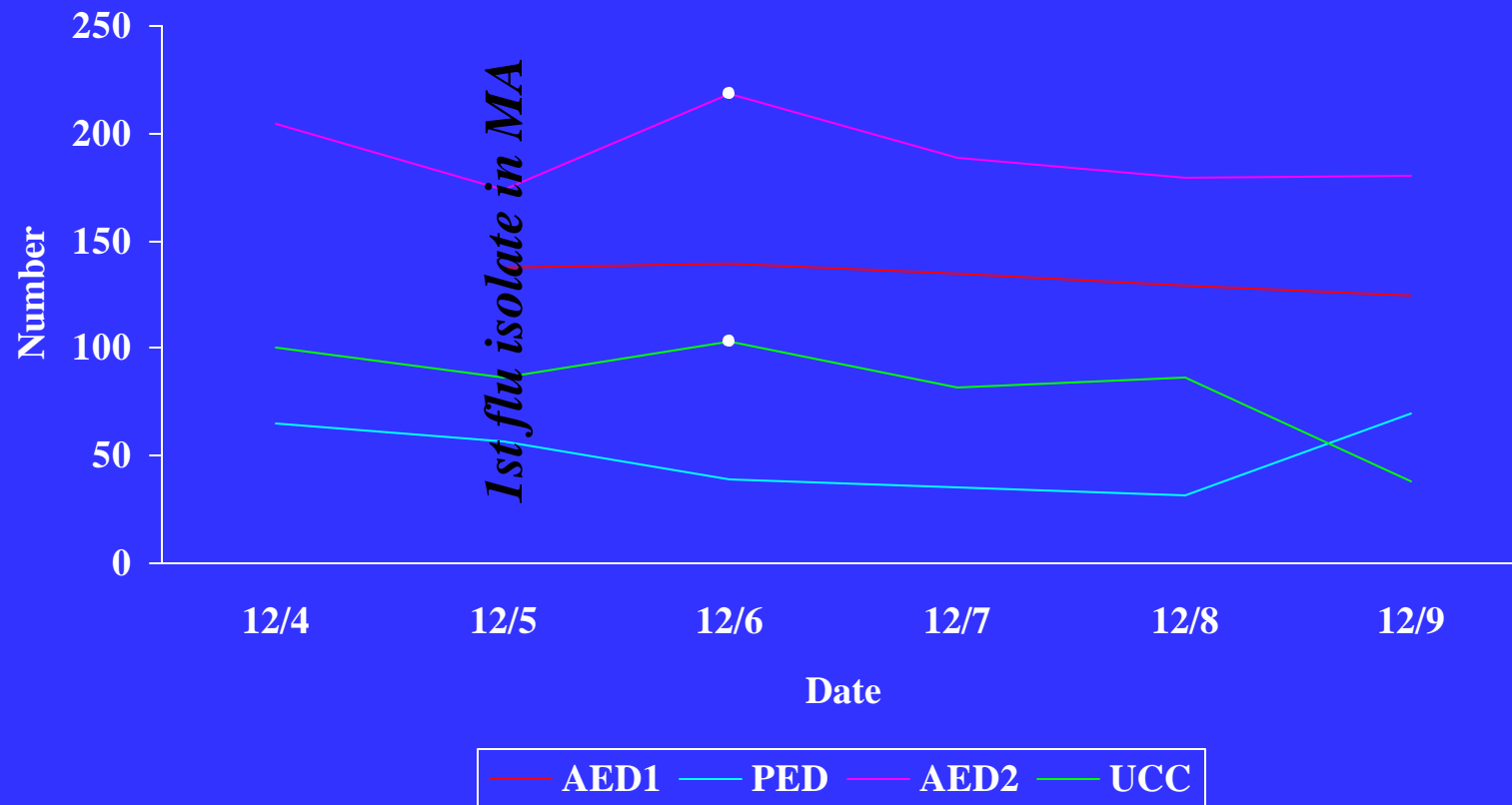
Daily volume by site

December 1, 1999 - January 31, 2000



- Days exceeding threshold
- Peak influenza activity in the U.S. (12/26 to 1/15/00)

Volume Surveillance - 12/4 to 12/9/00

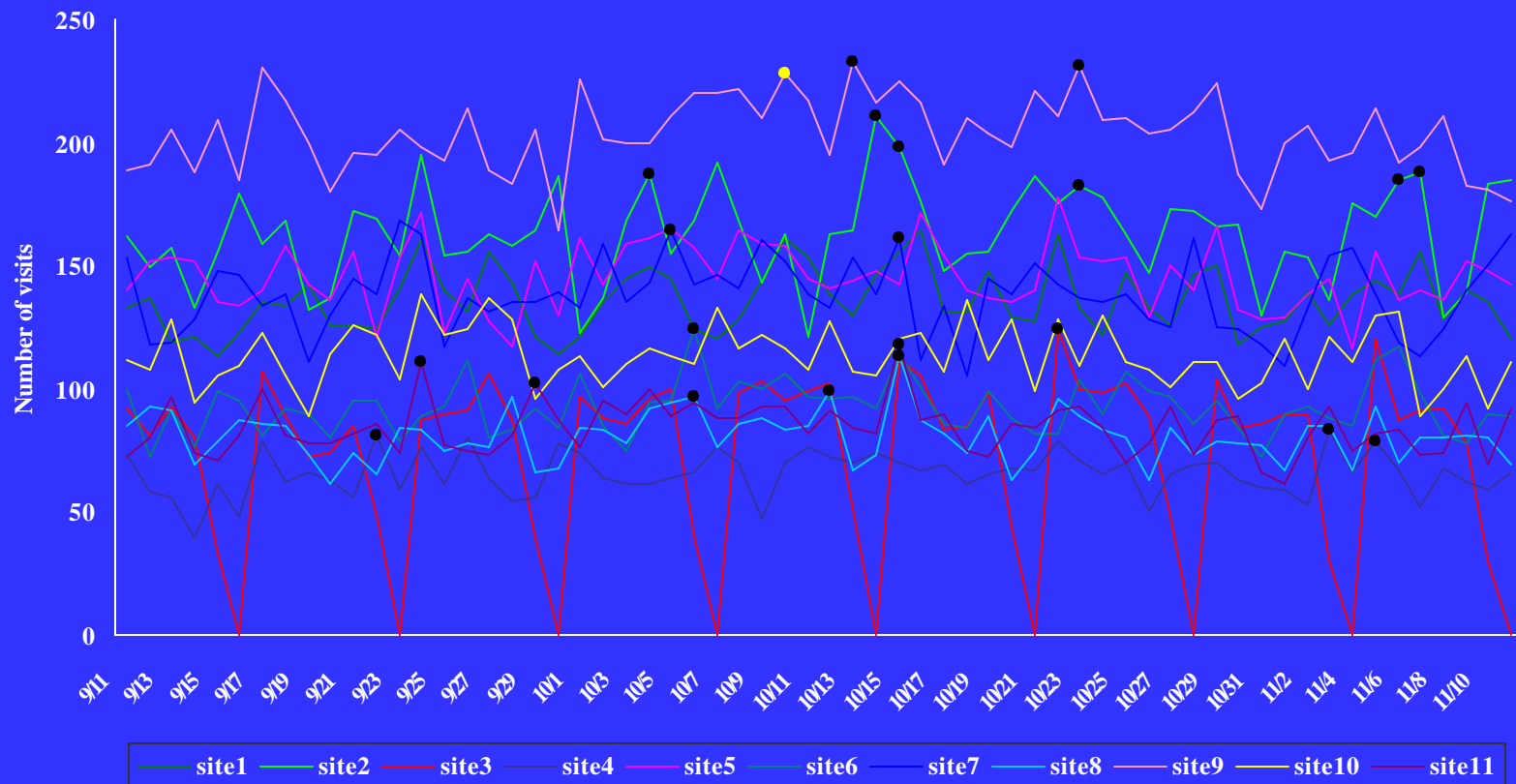


- Exceeded threshold

Volume data: Findings from 9/11/02 -11/11/02

Daily volume by site

September 11, 2001 - November 11, 2001



?Days exceeding threshold. No infectious disease clusters identified.

How many times did multiple sites exceed threshold on a given day?

- There were 22 episodes of a site exceeding threshold in the time period.
- For most (n=17) only a single site exceeded threshold on a given day.
- On two days, two sites simultaneously exceeded threshold.
- On one day, four sites simultaneously exceeded threshold.

Follow-Up with sites exceeding threshold and Boston Public Health Commission's (BPHC) Response

- Persons seeking nasal swabs and antibiotics for anthrax resulted in increased activity on 10/15
- No anthrax cases or anthrax contaminated environmental specimens were identified in Massachusetts
- The BPHC posted information on anthrax including updates to BPHC's website (www.bphc.org)
- Clinical advisories on anthrax were emailed to health care providers throughout the city

Enhanced Surveillance in Boston

Strengths

Adjusts for site case mix

Adjusts for seasonal changes

City wide coverage

Electronic

Weaknesses

Non-specific for BT events

Changes influenced by the business of health care

Conclusions

- Volume based surveillance is a feasible method for the early identification of a mass morbidity event
- A rapid follow-up system is a critical component to understanding initial signals
- Data from this system can be used to create educational messages for both health care providers and the public
- Additional research is needed to define the sensitivity of the individual or combined measures being used and the optimal combination to detect significant activity

Enhanced Surveillance in Boston: Lessons Learned

- Systems must be electronic
- Add on systems will not be sustainable
- Computers system go down (even for days)
 - Develop back up plans
- Don't abandon case reporting
 - No one system is perfect
- The more complex data - the harder it will be to retrieve it manually
- Build communication networks into the surveillance system

Enhanced Surveillance in Boston: Future Plans

- Capture more granular data
 - Chief complaint data
 - Natural language programming
 - Minimize human contact
- Add additional populations and types of health care sites
- Enhance the surveillance feedback loop
- Syndromic surveillance

Syndromes That May Be Associated With Bioterrorism

- **Pulmonary**
 - **Fever**
 - **Cough**
 - **Myalgias**
 - **Hypoxia**
- **GI**
 - **Fever**
 - **Nausea/vomiting**
 - **Diarrhea (+/-bloody)**
- **Rash and fever**
 - **Vesicular**
 - **Petechial**
- **Neurologic**
 - **cranial nerve palsies, HA, fever, confusion**
- **Septic Shock**
 - **DIC**
 - **Organ failure**

Syndromic Surveillance

- ICD-9 code data or chief complaints to identify potential BT-related syndromes
 - How much is to much
 - Follow-up is critical
 - Real time data is limited
 - Sustainability
 - Validity of chief complaint data - How do different populations describe illness

Questions?



Bioterrorism Preparedness - Laboratory Analysis

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Bioterrorism Preparedness - Laboratory Analysis

An account from
the “real world”
of the clinical
microbiology
laboratory



Clinical Laboratories - The Need for Preparation

- Agents likely to be used by terrorists
 - Unfamiliar, rarely encountered organisms
 - Potential for misidentification, mishandling of specimens, laboratory acquired infection
- Public health agency-sponsored training in the Northeast began in 1999
- Laboratory Response Network (LRN)
- Were we prepared in the autumn of 2001?

Autumn, 2001 - Anthrax!

- Wake-up call for clinical microbiologists
- Expect the unexpected
- Preparedness is an absolute necessity

LAB LABEL

Outpatient Requisition Clinical Laboratories

Date Collected: _____ Time Collected: _____ Phleb: _____

ORDERING PHYSICIAN NAME: _____ PROVIDER# _____

Copy to Physician Name: _____ Specimen Type/Site: *throat swab*

ICD - 9 CODES: _____ PROVIDE DX IF CODE UNKNOWN: *cough paric disorder*

Attention: All services ordered for the patient must meet the definition of medical necessity (i.e., required to diagnose or treat an illness or injury). Documentation must be sufficient to demonstrate same.

REFER TO BACK FOR PARTIAL DIAGNOSIS LISTING

r = 5mL red B = 10mL red c = 5mL (green/gel) G = 5mL grey (half-full) L = 3mL lavender b = 4.5 mL blue u = urine

MICROBIOLOGY SECTION

(Susceptibility performed when required)

☐ Beta Strep Culture, throat only

☐ Cervical Culture

☐ Chlamydia (Body Site required: _____)

☐ Gonorrhea (Body Site required: _____)

☐ Herpes (HSV), (Body Site required: _____)

☐ Heterophile Antibody (Monospot)

☐ Ova & Parasites

☐ Stool Culture

☐ Urine Culture

☐ Vaginal Culture

☐ Measles Ab

☐ RPR (serology)

☐ Rubella Ab

☐ HIV Viral Load

☐ Other: _____

☒ Other: *ANTHRAX*

Comments: *Sony!*

LRN Level A Lab Preparedness

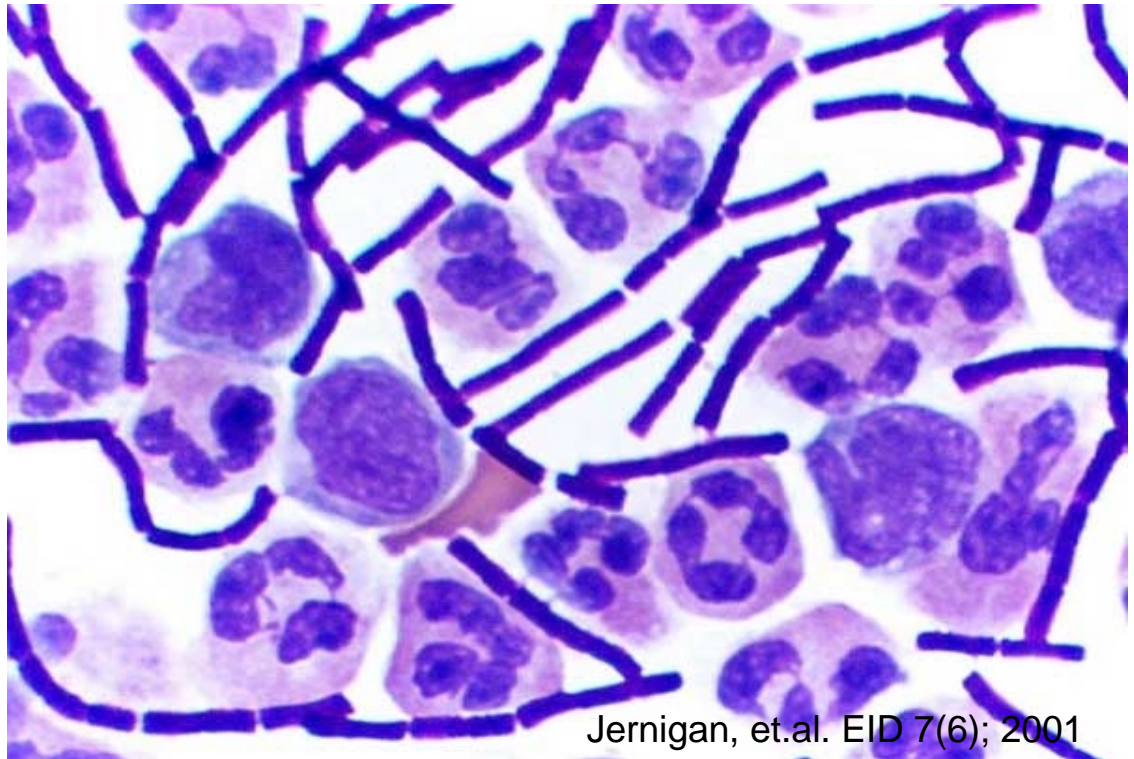
- Level A laboratory functions
 - Rule out / refer
 - Ship suspicious infectious agents to higher level labs for further study
- Level A laboratory activities
 - Formulate laboratory procedures
 - Train staff
 - Biosafety concerns
- Assistance from public health agencies

Activities of Clinical Micro Labs

- “Average” Labs
 - Microscopic examination of specimens
 - Culture of specimens and isolation of many bacterial and fungal pathogens
 - Identification and susceptibility testing
- “Advanced” Labs
 - Viruses (culture, direct detection)
 - Mycobacteria (culture, susceptibility)
 - Certain fungi (culture and identification)
 - Molecular testing

Level A Lab Example: *B. anthracis*

- Gram stain* of CSF, positive blood culture or wound culture shows large gram-positive rods



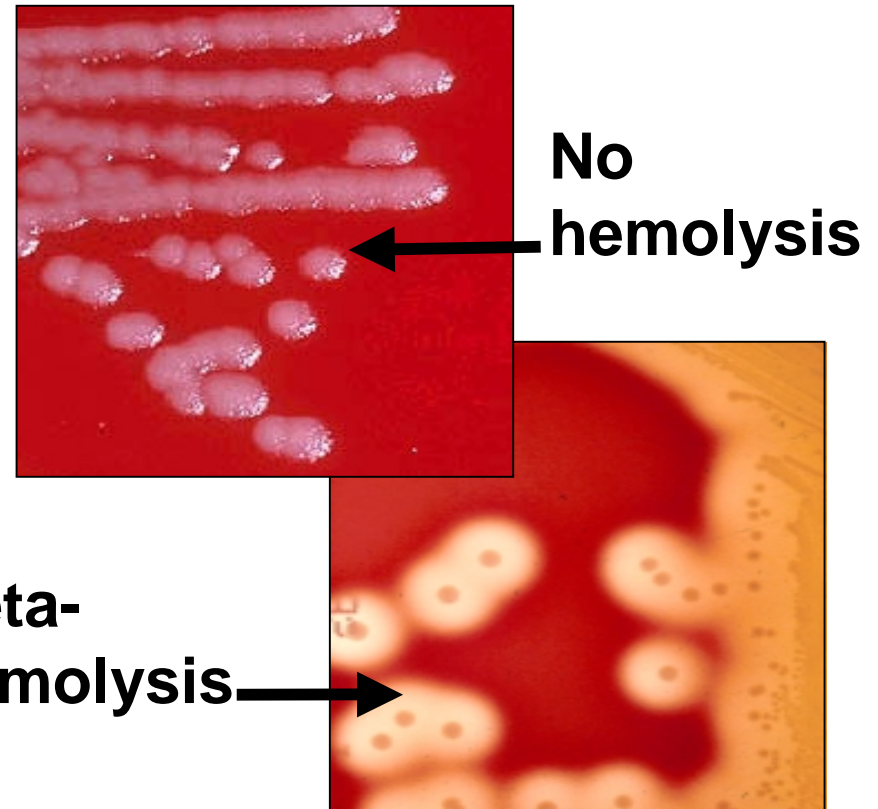
Jernigan, et.al. EID 7(6); 2001

*Gram stain: Differential stain, not specific, but can be extremely helpful



Level A Lab Example: *B. anthracis*

- Culture on blood agar*. Examine for characteristic colony morphology and lack of beta-hemolysis



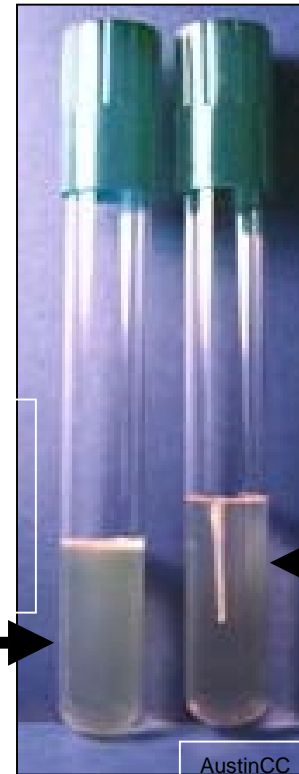
*Agents of anthrax and plague are “easy” to grow. Agents of tularemia, brucellosis are harder to recover, may require special media



Level A Lab Example: *B. anthracis*

- Perform identification tests. For ?*B. anthracis*, perform motility test*

Growth throughout medium (motile)



Growth only near original inoculation stab (non-motile)

*Minimal rule out tests (minimal manipulation of potentially dangerous cultures) are recommended for Level A labs



Level A Lab Example: *B. anthracis*

- Ruled in?
 - *Bacillus* species with characteristic colony morphology, non-hemolytic, non-motile
- REFER
 - Contact Level B lab
 - Ship suspect isolate



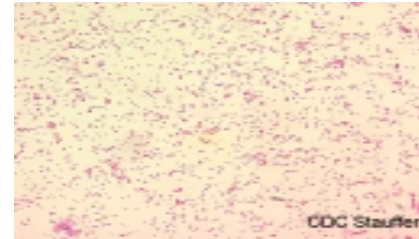
Level A Lab Preparedness - Where Are We Now?

- Bigger seems to be “better”
 - Wider variety of pathogens encountered; personnel experienced in working with infrequently isolated agents
 - More and/or better biosafety equipment
 - Institutional support for needed resources is more likely in larger hospitals
- Small labs can still have successful preparedness programs

Level A Lab Preparedness



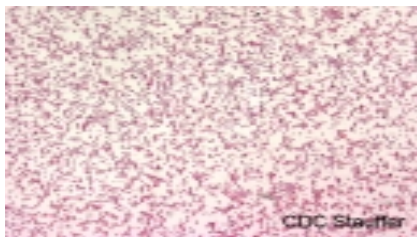
Anthrax



Brucellosis



Plague



Tularemia



**Botulism-
Specimen processing/
shipping only**



**Smallpox, VHF-
More guidance needed
for Level A labs**



**Environmental testing
for *B. anthracis* spores**

Clinical Lab Preparedness – Next Steps

- Extend training (category B agents)
- Enhance communication/cooperation with higher level public health labs
 - NLS
- Dissemination of some Level B procedures to select Level A labs
 - ?Rapid, specific tests/reagents
 - ?BSL3 activities in select labs
 - ?Surge capacity

Level A Clinical Microbiology Laboratories

- Can be instrumental in early recognition
- Must be trained, alert and vigilant
- Form partnerships with public health labs for BT preparedness assistance, BT response plans, and overall improvement of the public health system

Urban Testbed Initiative

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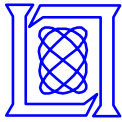
3 April 2001

MS-15433

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years





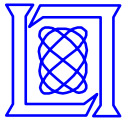
Outline

- **Thoughts on Urban Biodefense**
- **Importance of Testbeds**
- **MIT LL Urban Testbed Initial Approach**
- **MBTA subway experiments**
- **Algorithmic approach**
- **Future Work**



Challenges Associated with Civilian Biodefense

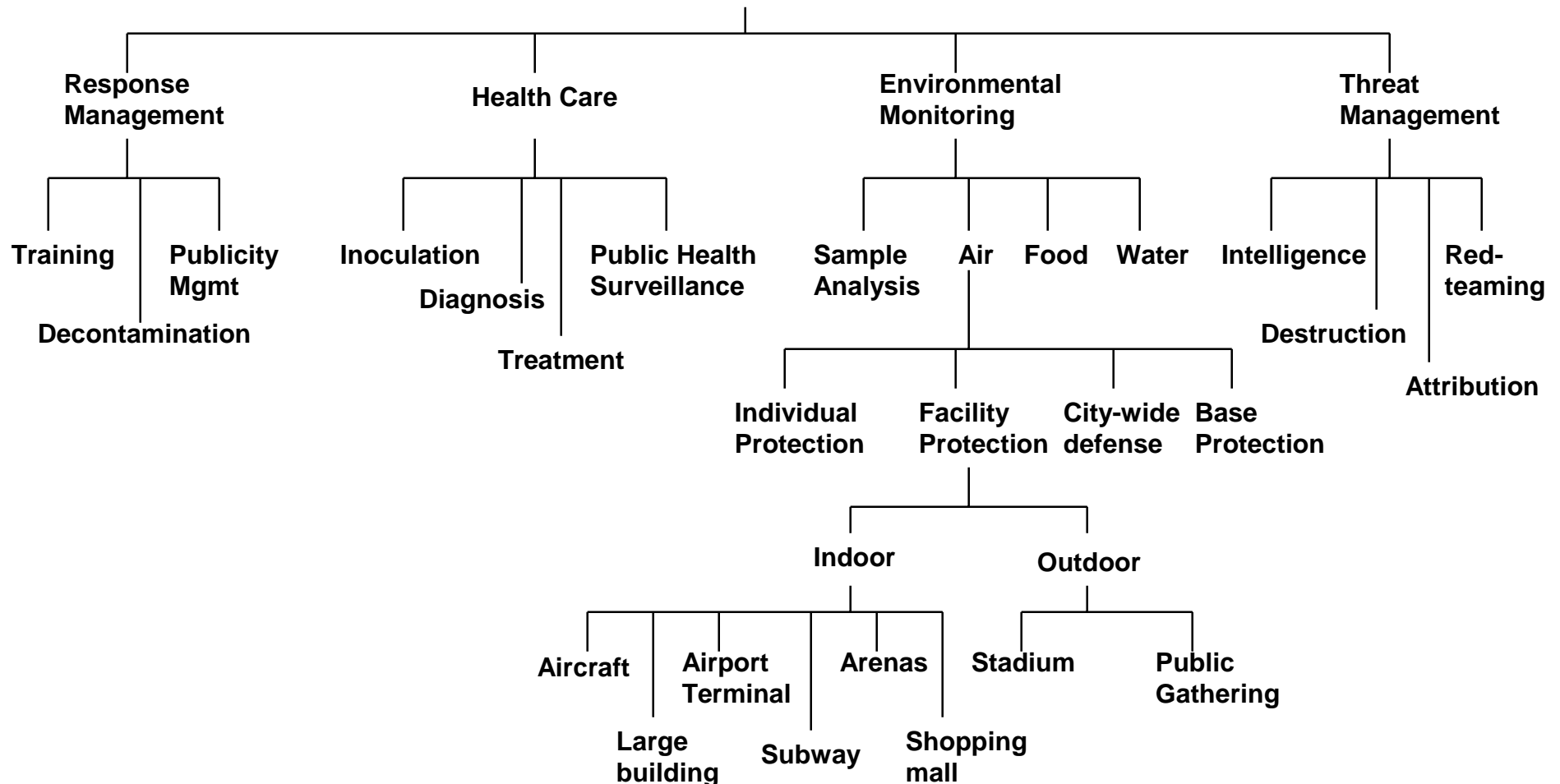
- **Any high-density site (city, airport, facility, building) represents a potential target**
- **Population to be protected is diverse (age, mobility, health)**
- **No environmental sensing systems will be tolerated that have high false negative or false positive rates**
 - **If they alarm too much or miss events, they will be ignored**
- **Current clinical diagnostic technologies and medical infrastructure are not suited to rapid detection of bioagent events**
 - **Advanced diagnostics (e.g., PCR) use is rare, even in large city hospitals**
 - **No medical reporting systems are in use that have real-time detection of infectious disease patterns as their objective**



Biodefense Components

Homeland Biodefense

*Numerous relationships between
portions of this hierarchy are not shown.*



Biodefense development must be multi-faceted.



Needed Biodefense Investments

- **Point-of-care and public health not well integrated**
 - Health care system is the current detector
- **System (multi-sensor) environmental monitoring development**
 - Focus has been on basic technology and devices
- **Characterization of environments of high-threat facilities**
 - Sensor technology not universally applicable
 - Helps to set requirements
- **Large-scale urban protection**
 - Sparse sampling/sensing
 - Low probability event with catastrophic consequences (akin to nuclear detonation)
- **Red-teaming**

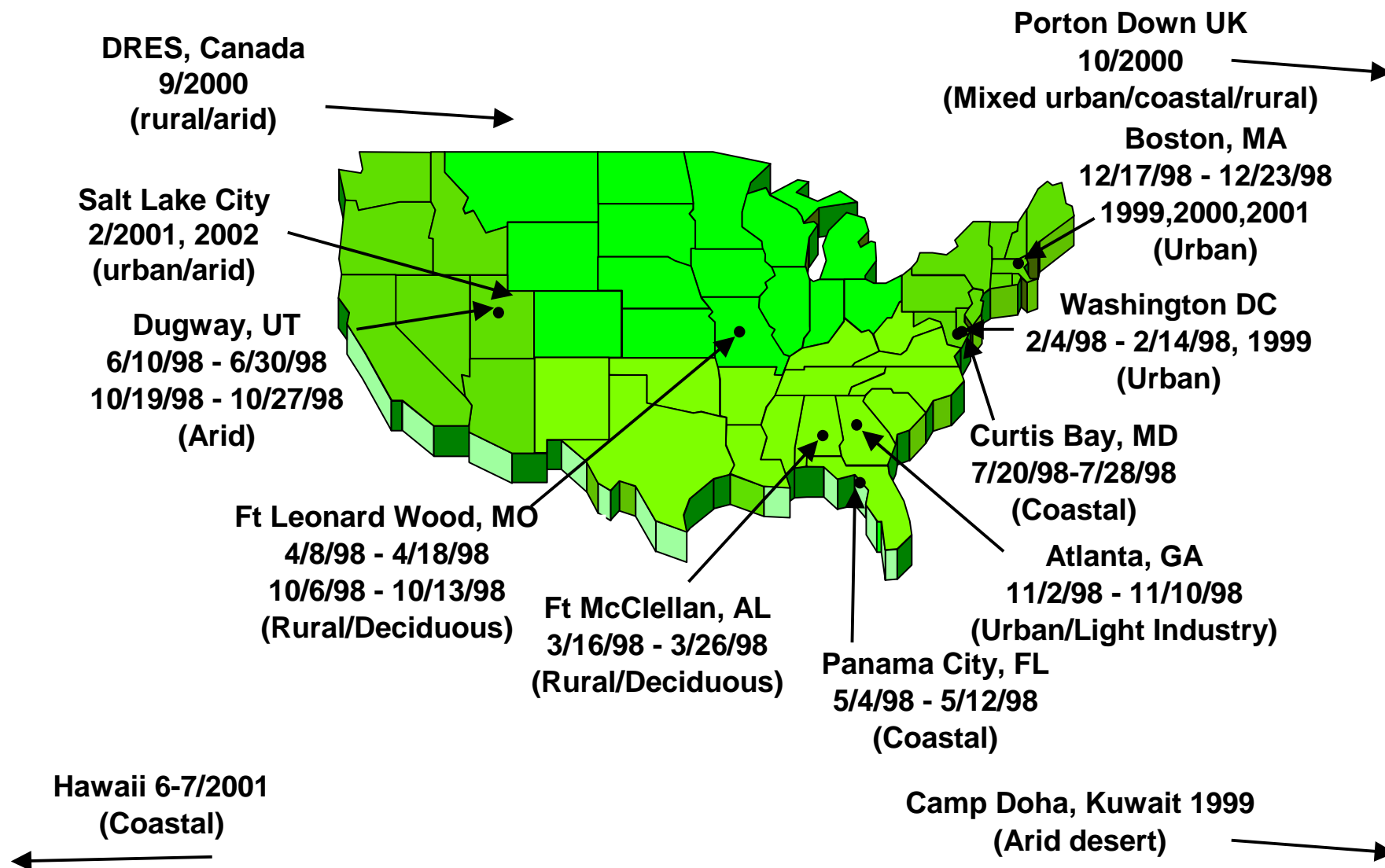


Environmental Monitors

- **DoD environmental monitors designed for outdoor force protection**
 - High sensitivity preference
 - Current cost prohibits mass-production
 - Unproven performance in urban or indoor areas where air is filled with interferents
- **Urban Civil Protection has markedly different requirements from military use**
 - Low false alert rate and low cost a priority
 - › Lower sensitivity partial solution may be preferred
 - Wide variation in environments (e.g. stadium vs. subway)
 - › Densely populated areas add to natural biological interferents
 - › Airflow, HVAC are important design considerations

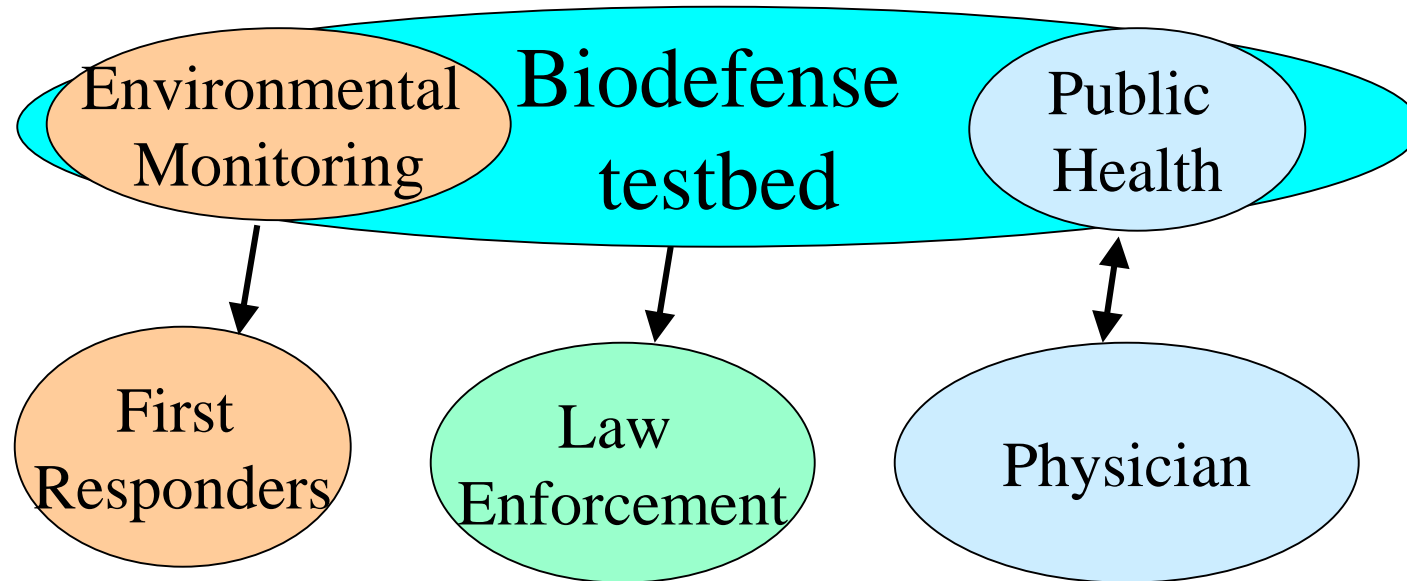


BAWS III Background Measurement Campaign





Testbeds as an Important Development Tool

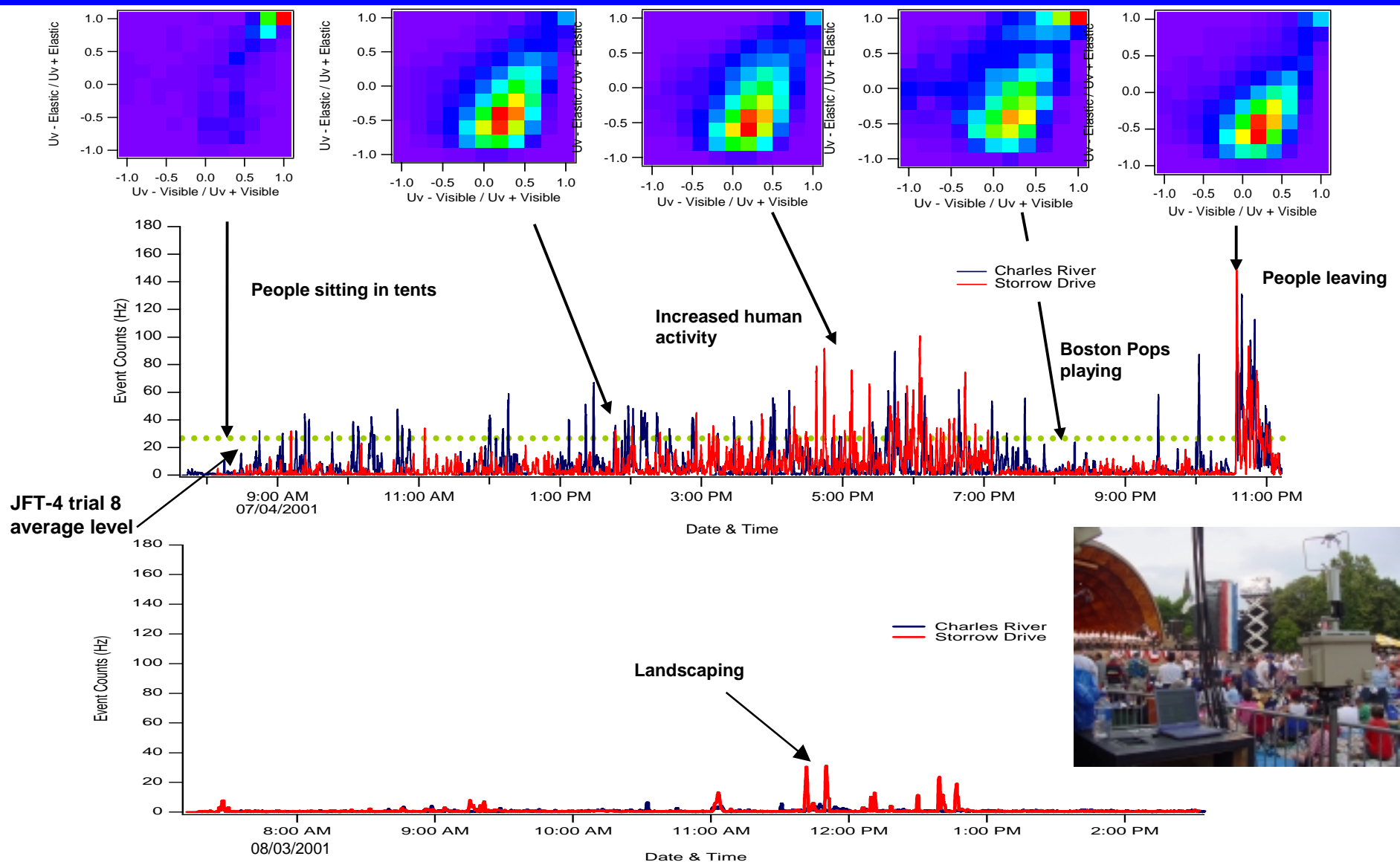


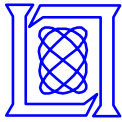
Testbeds are needed for both public health and environmental monitoring systems

- Understand the problem and set system requirements
- Improve training
- Infuse emerging technologies in realistic settings
- Understand unique environments of various facility types



BAWS July 4, 2001 Esplanade Measurement





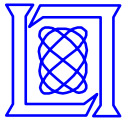
MIT/LL Urban Testbed Project Goals

- **Define a system architecture for facility defense using environmental monitors**
- **Understand the natural air composition and the response of existing instruments in those facilities**
- **Develop decision logic methodology that is extensible to other urban defense problems**



Urban Testbed Status

- **Project funding began in June, 2001**
- **Coordination with Boston-area authorities for the past 1-2 years**
 - **MA Bay Transportation Authority (MBTA), Boston Emergency Management Authority (BEMA), MA Emergency Management Authority (MEMA), MA Dept of Public Health, National Guard, Logan airport, others**
- **BAWS measurements at Boston Marathon, July 4th celebration**
- **Measurements in MBTA subway station; sensors being installed in a station.**
 - **Particle counters, airflow, temperature, humidity, train motion.**
 - **Periodic measurements in other locations or with sensors that cannot be installed for long periods.**
- **Develop alerting algorithm approach**
- **Controlled chamber releases**
- **Discussing measurements in other Boston locations**



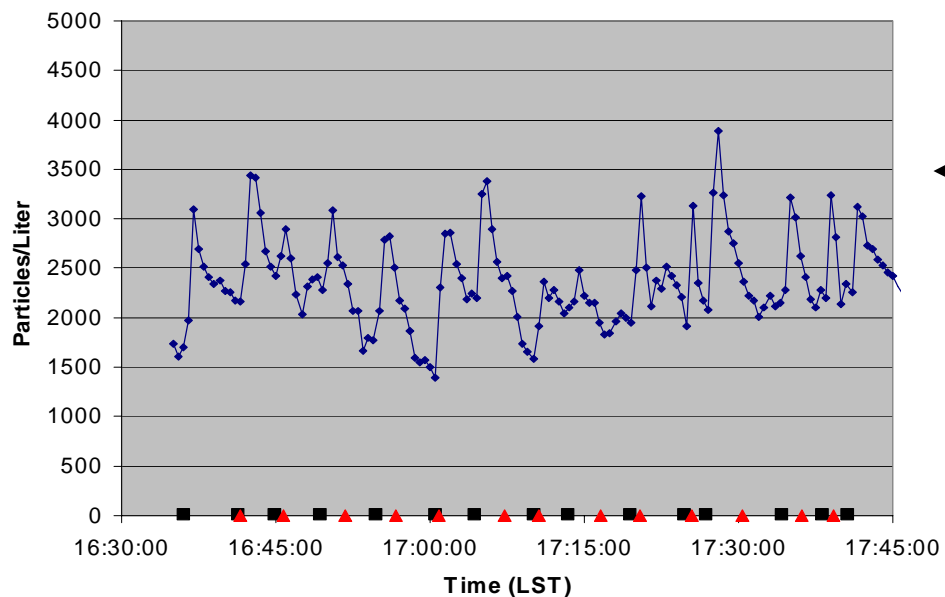
Subway Protection Considerations

- **Threat has been established**
 - Aum Shinrikyo Tokyo Sarin gas release
 - Numerous entry points and hiding places
 - Train “piston effect” moves air through the system
- **System is spatially distributed**
 - Many low cost sensors preferred over few high cost sensors
 - Release point cannot be anticipated apriori
- **Important to find dual-use applications for system**
- **Principal response actions**
 - Stop trains (plug tunnels?)
 - Activate vent fans?
 - Evacuate and prevent additional access



Station Particle Counts

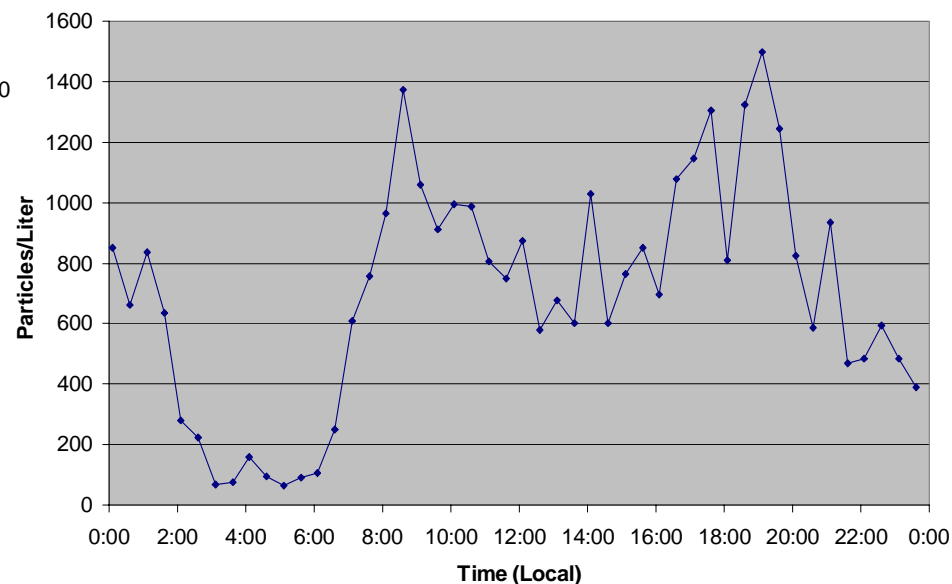
Particle Counts for 2-5 micron region



Train Traffic significantly alters particle counts

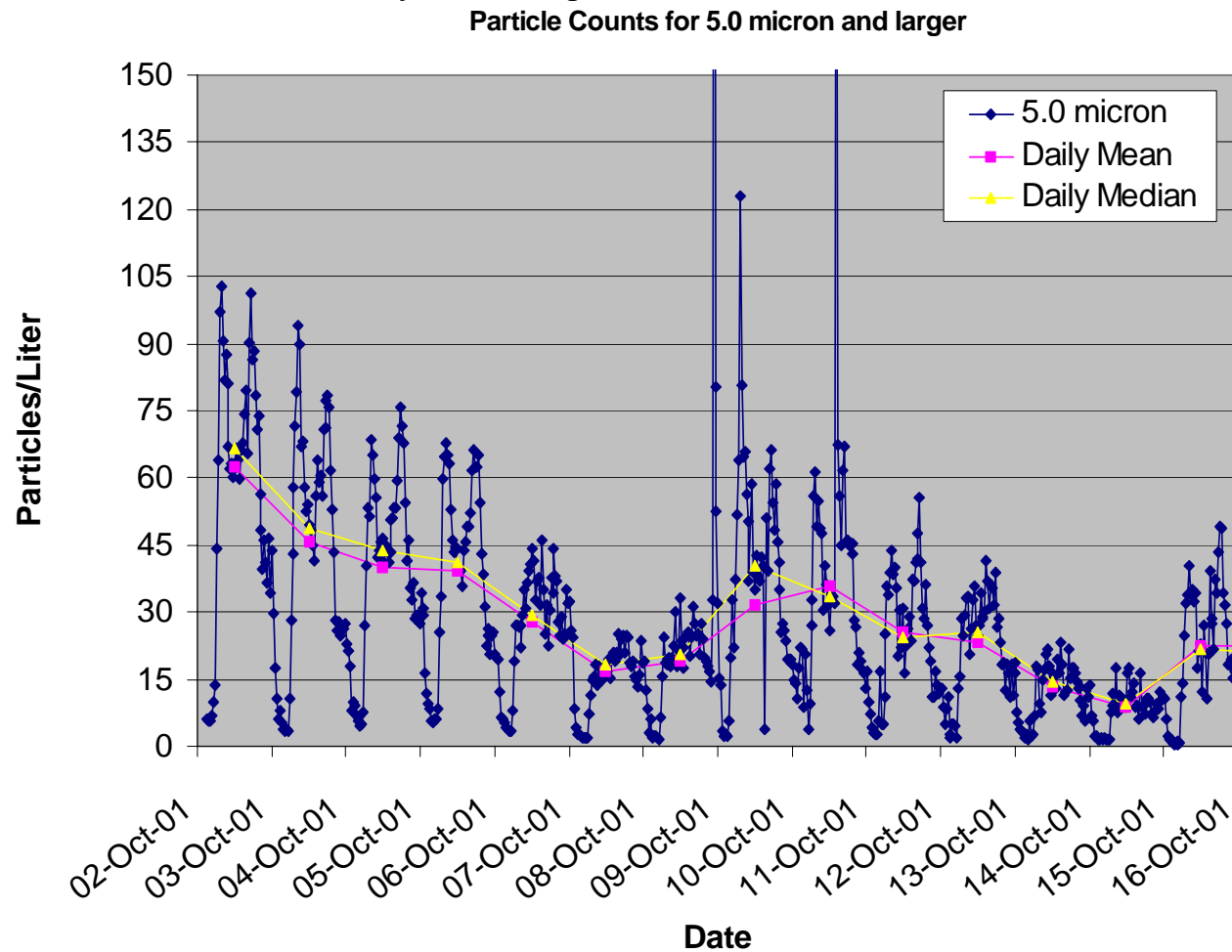
Diurnal Cycle significantly alters particle counts

Particle Counts for 2-5 micron region

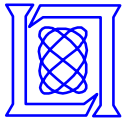




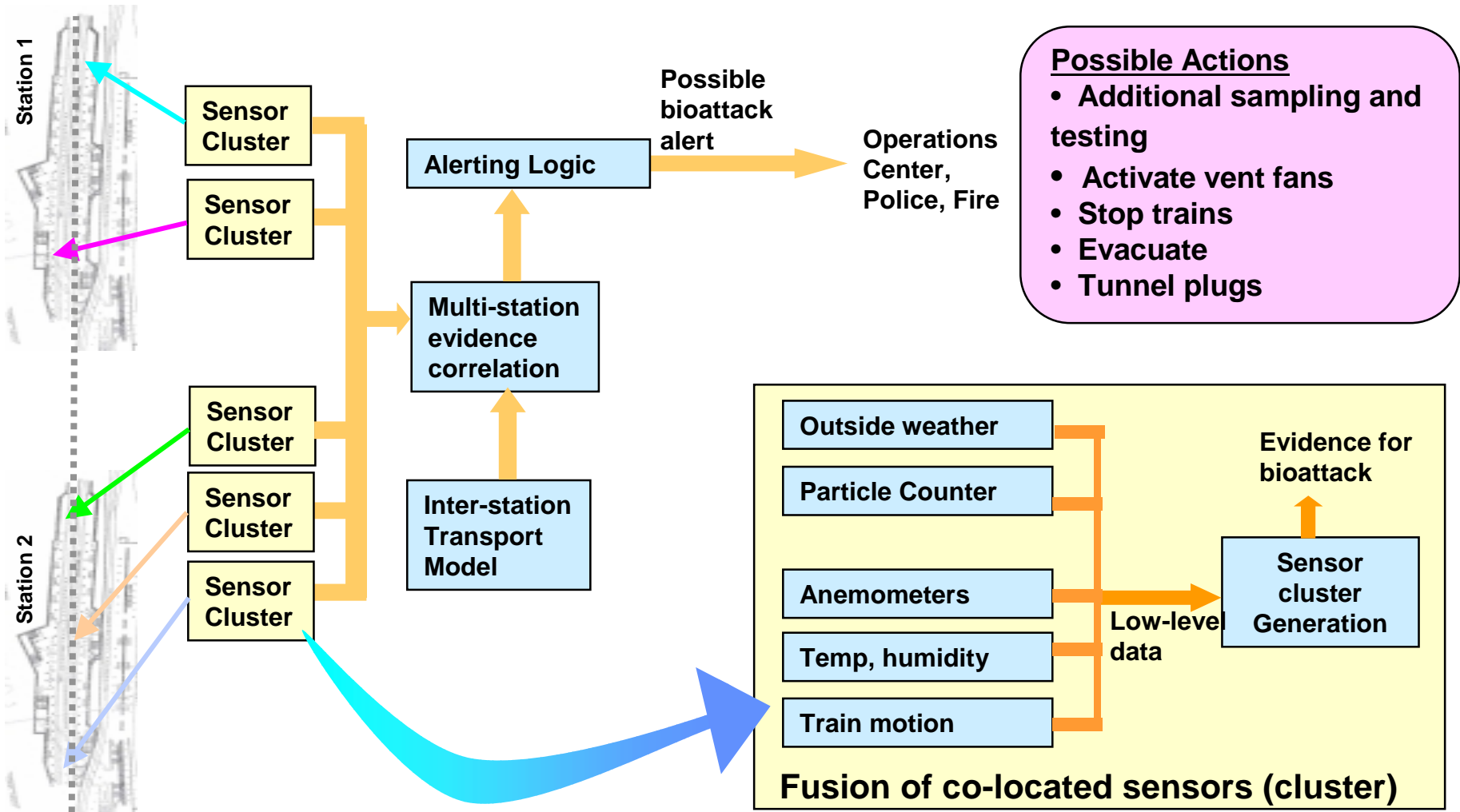
Subway effects on Particle Counter Sensors



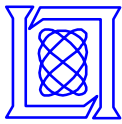
**Particle counter sensors degrade quickly due to laser optics contamination.
Full instrument sensitivity regained after cleaning.**



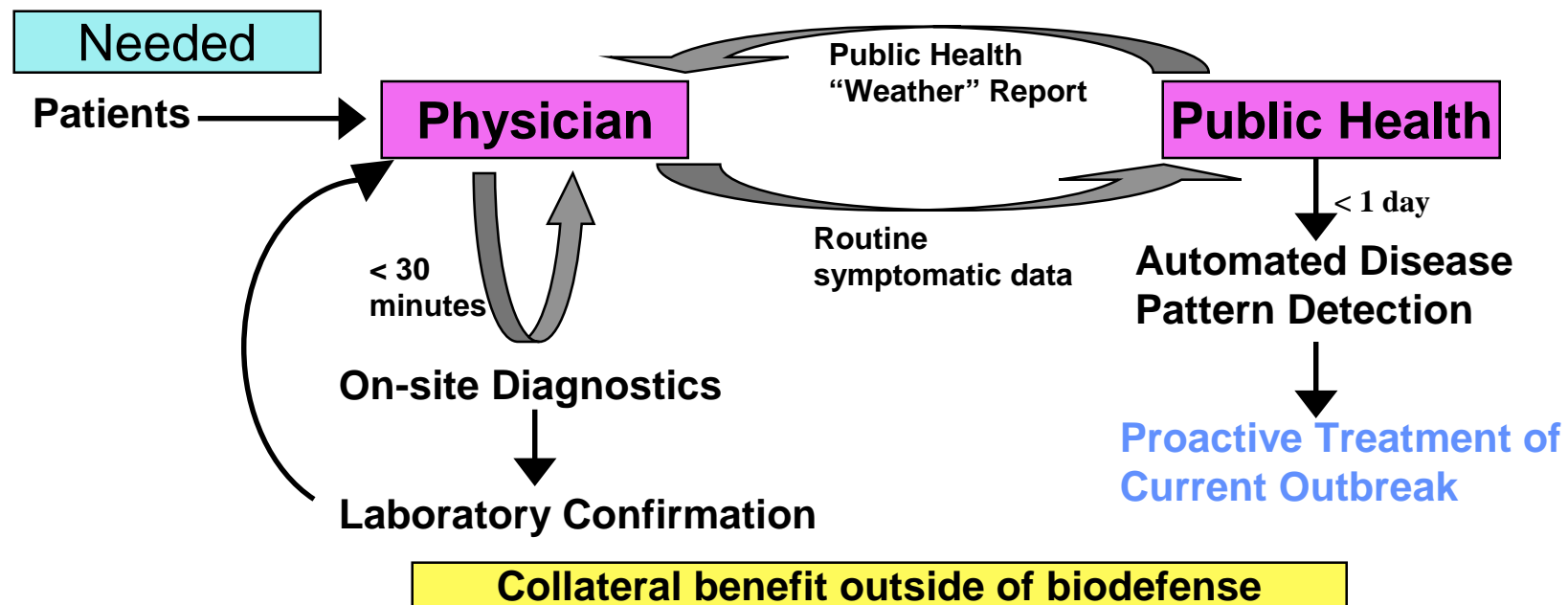
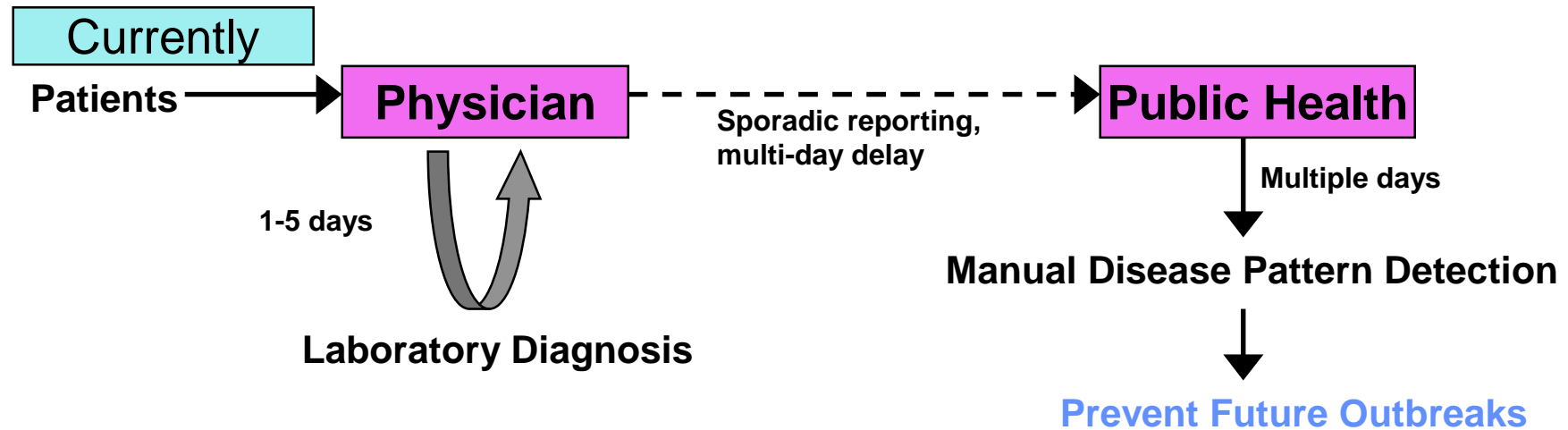
Subway Alerting Algorithm Architecture



Multiple sensors required to agree and sensitivity reduced to reduce risk of false alert.



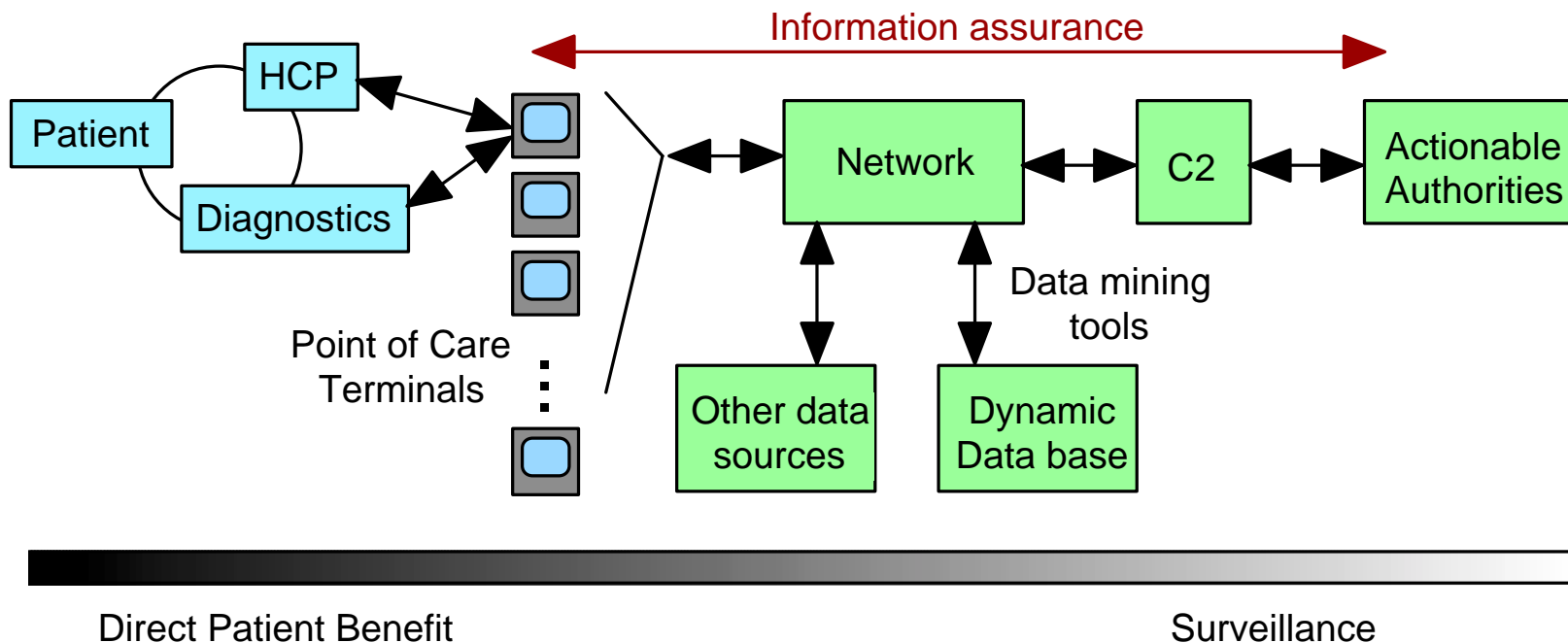
Health Care Provider and Public Health Integration

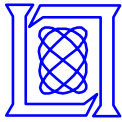




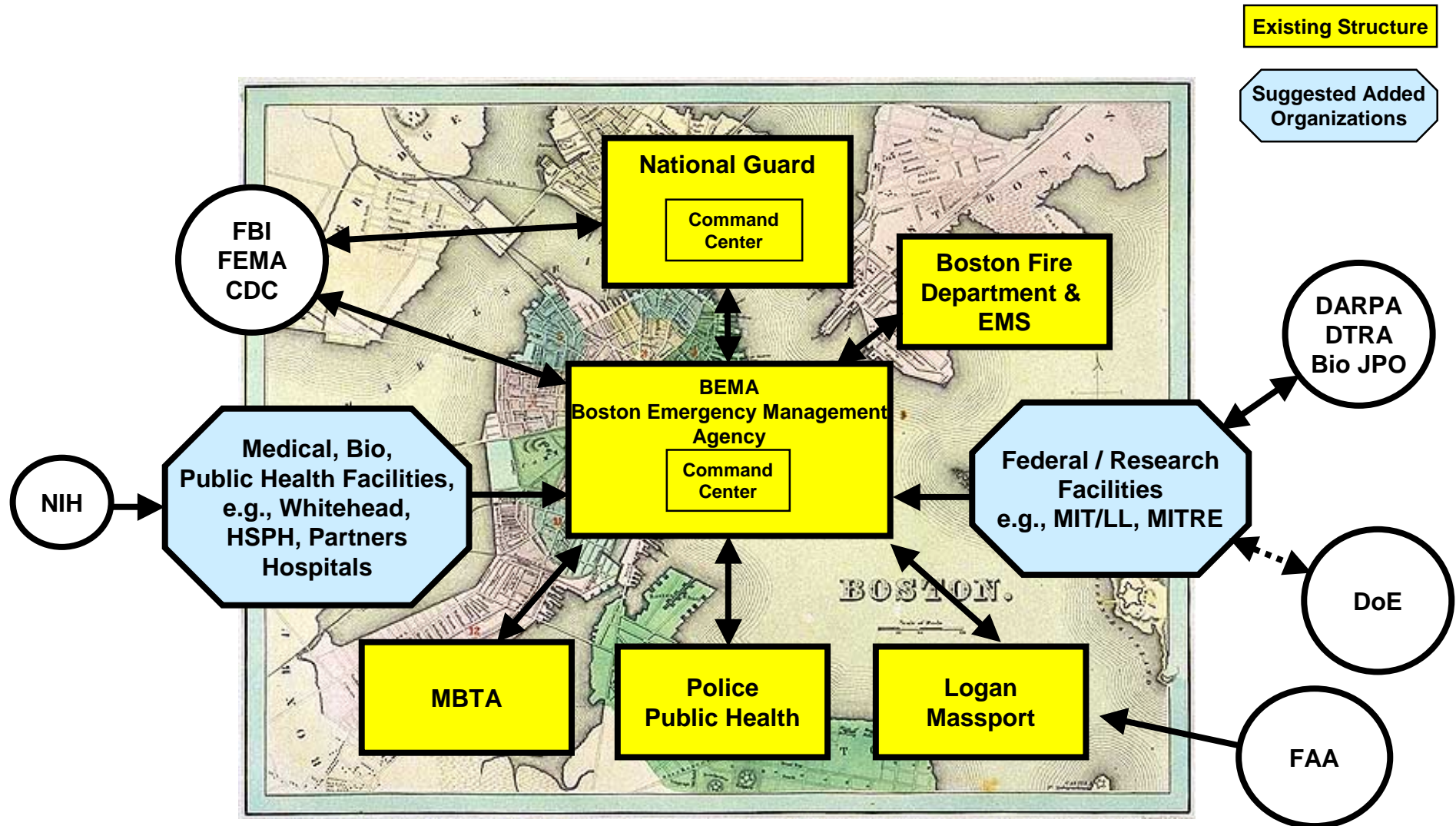
Merging of Health Care with Defense Against Biological Weapons

- **Concept:**
 - Implement advanced point-of-care diagnostics (including but not limited to gene-chips), into IT networked system
 - Enables rapid determination of biological attack
 - Benefits natural infectious disease diagnosis, effective treatment





Boston Area Agencies with Biodefense Responsibilities





Summary

- **Civilian bioterrorism defense requires that the environment of high-threat locations be well understood**
 - Environment drives sensor & system design
- **Initial testbed being installed at Boston subway station**
- **Measurements to date point out deficiencies of current sensors & software**
- **Modern recognition/data fusion techniques being applied to data**
- **Measurements at additional Boston threat locations under discussion**

Facility Defense Against Aerosol Attack

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3 April 2002

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years



MS-15434

This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.

MIT Lincoln Laboratory



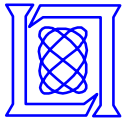
Outline

- **Facilities and attack scenarios**
- **Sensing an attack**
- **Facility protection techniques**

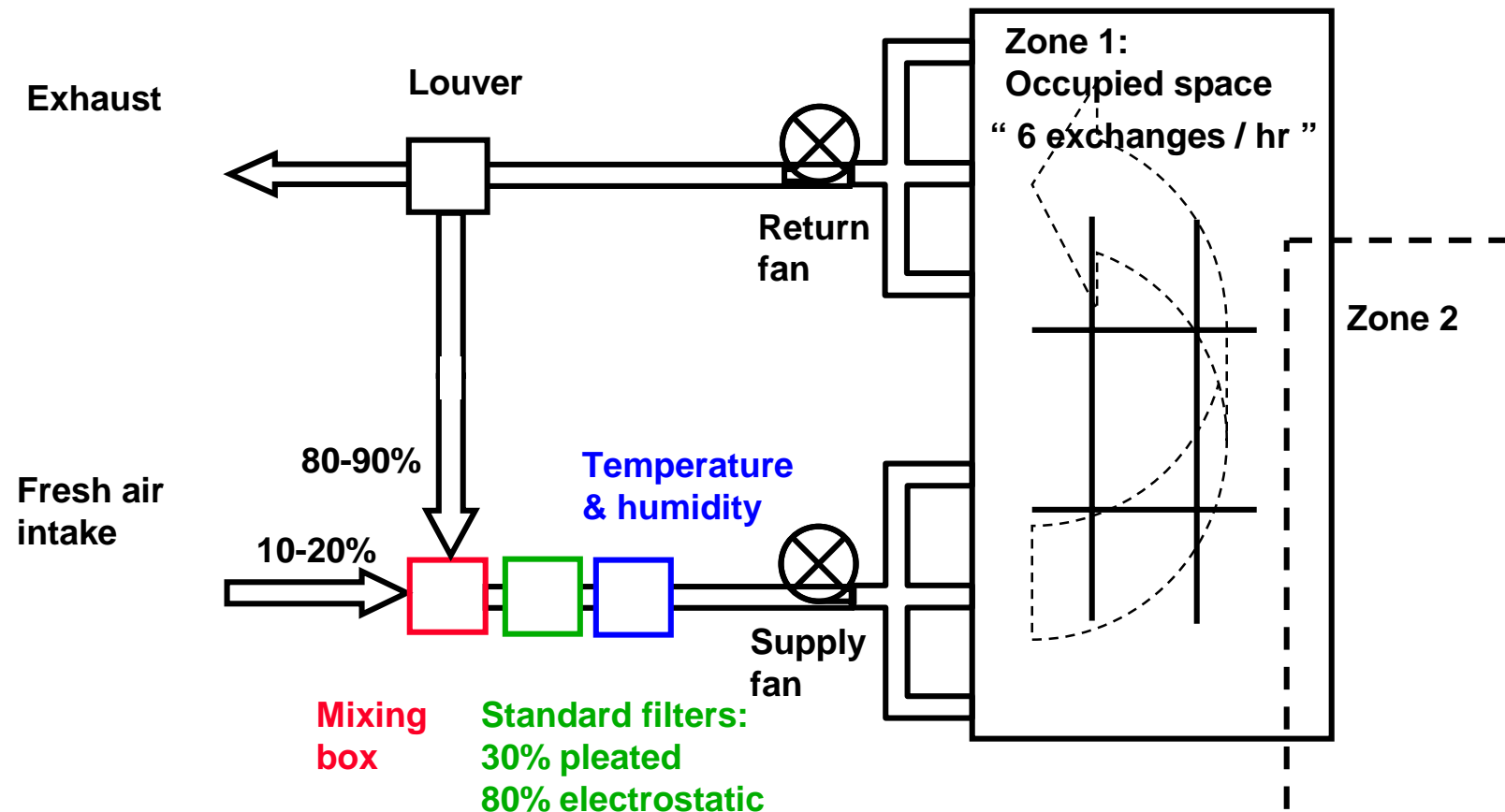


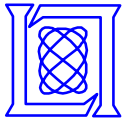
Types of facilities

- **Simple structures**
 - Residences, barracks
- **Buildings with ventilation system**
 - **Multiroom office building**
 - Large open space (arena, terminal, ...)
- **Subway**
- **Outdoor sites**
 - Stadium
 - Public gathering
 - Military operations



Simplified Ventilating System





Types of Attacks

- **External attacks**
 - Nearby cloud release
 - Burst release into air intake
- **Internal attacks**
 - Burst release into air return
 - Burst release into a large open space
 - Low level continuous release
- **Small amounts of agent are substantial threats**

1 gram bioagent uniformly dispersed into 10^8 liter building (100m x 100m x 10m);

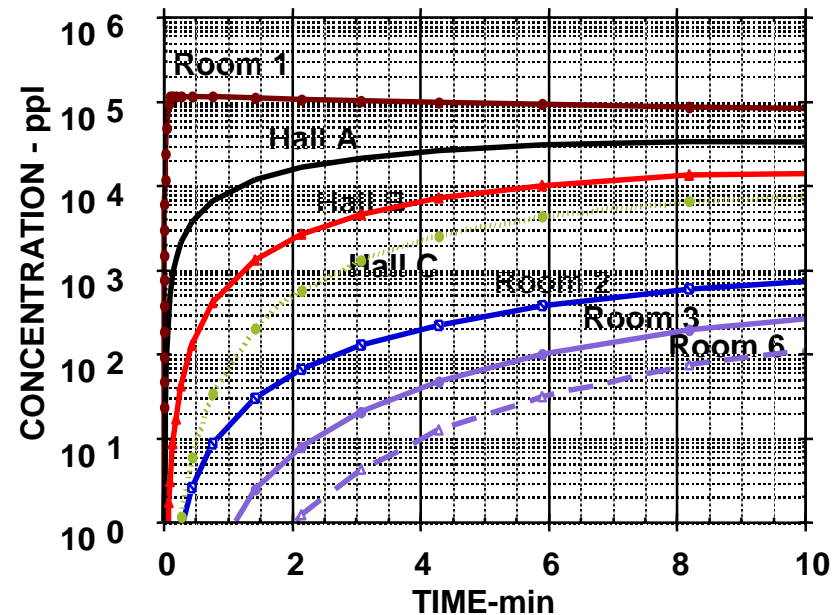
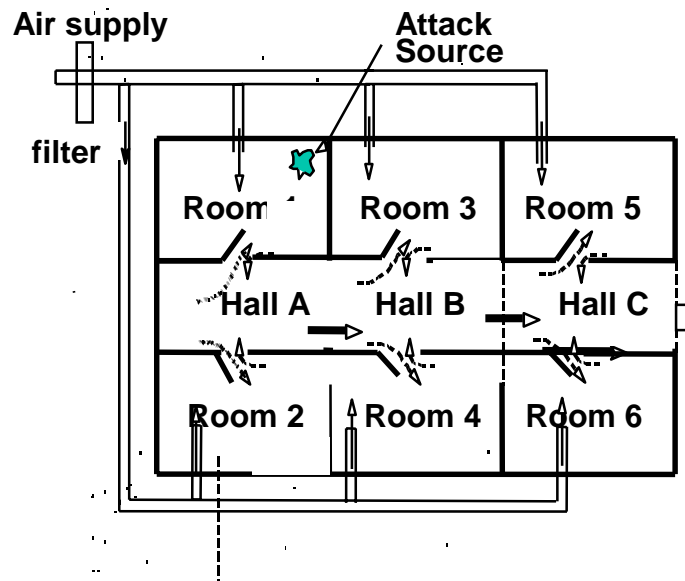
Corresponds to lethal exposure (100 ppl x 10 liter/min x 10 min; 10^{10} particles /gram)



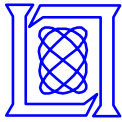
Modeling an Attack

Burst release in an interior room

- Bioagent - 15 grams over 5 sec
- Room-Hall coupling - 10%



- Lumped parameter models are well established
instantaneous and uniform concentration within each room
- Initial particle dispersal and deposition are more complicated to model.



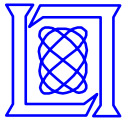
Emergency Management Measures

- **Information**
 - **Observing suspicious activity**
 - **Knowing who to treat**
 - › **Primarily, but not exclusively, bio agents**
 - › **Records of access (badge swipes, tickets,...)**
 - › **Voluntary response to public announcement**
 - › **Physical examination**
 - **Preserving forensic evidence**
- **Plan of action**
 - **HVAC emergency management decision tree**
 - › **Suspicious event near air intake -> shut down intake**
 - › **Suspicious event inside building -> full fresh air**
 - **Communication channels**
 - **Evacuation plan**
 - › **Orderly movement to controlled safe area, avoid cross contamination**



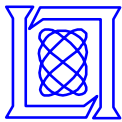
Outline

- Facilities and attack scenarios
- Sensing an attack
- Facility protection techniques

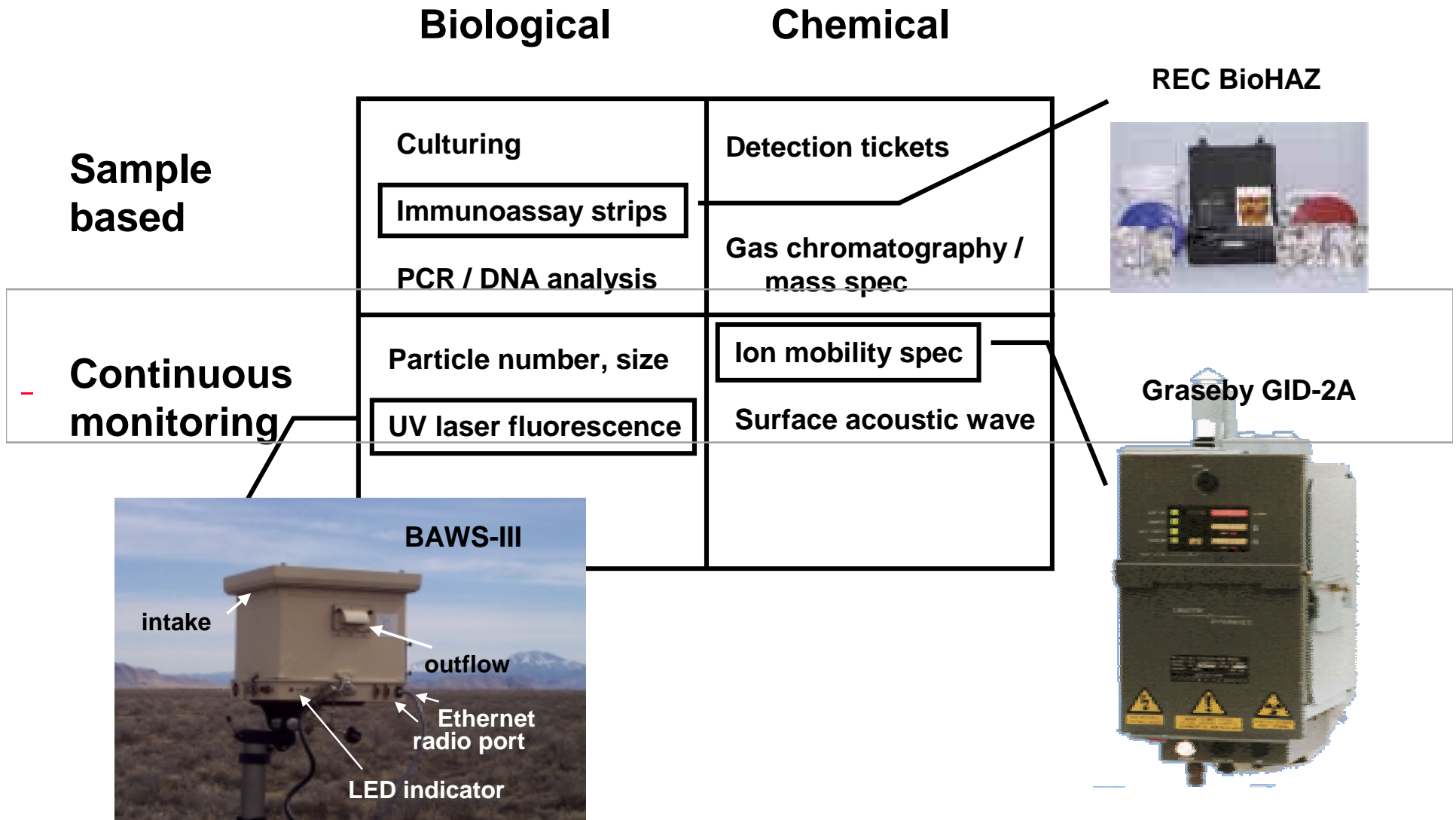


Rationale for Sensing

- **Issue alarm**
 - initiate facility response
 - high $\text{Prob}_{\text{detection}}$; low $\text{Prob}_{\text{false alarm}}$; wide range of agents
- **Identification of agent**
 - initiate medical treatment
- **Mapping of contamination zone**
- **Assessing decontamination (“all-clear”)**

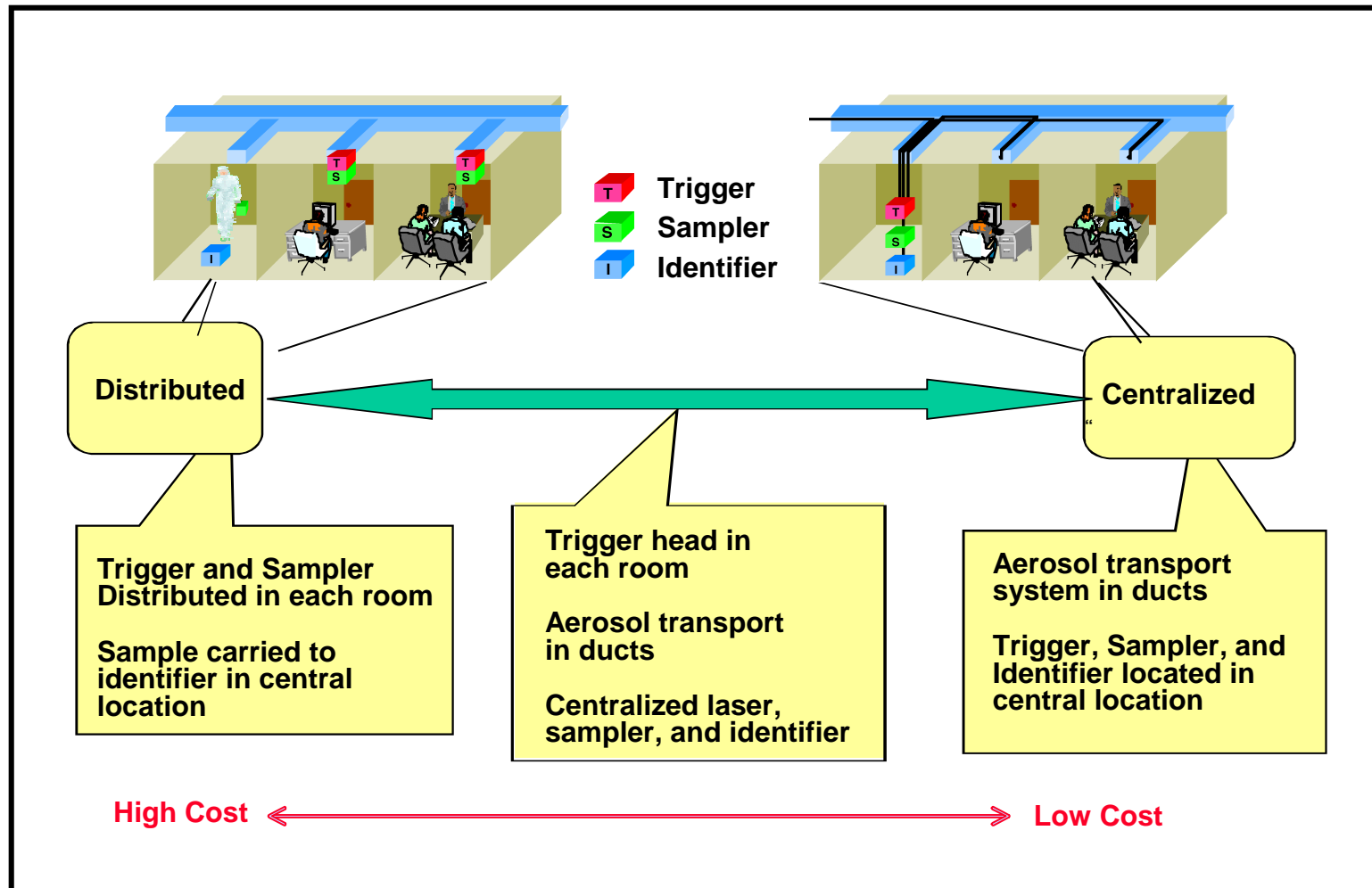


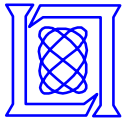
State-of-the-Art Bio / Chem Sensors



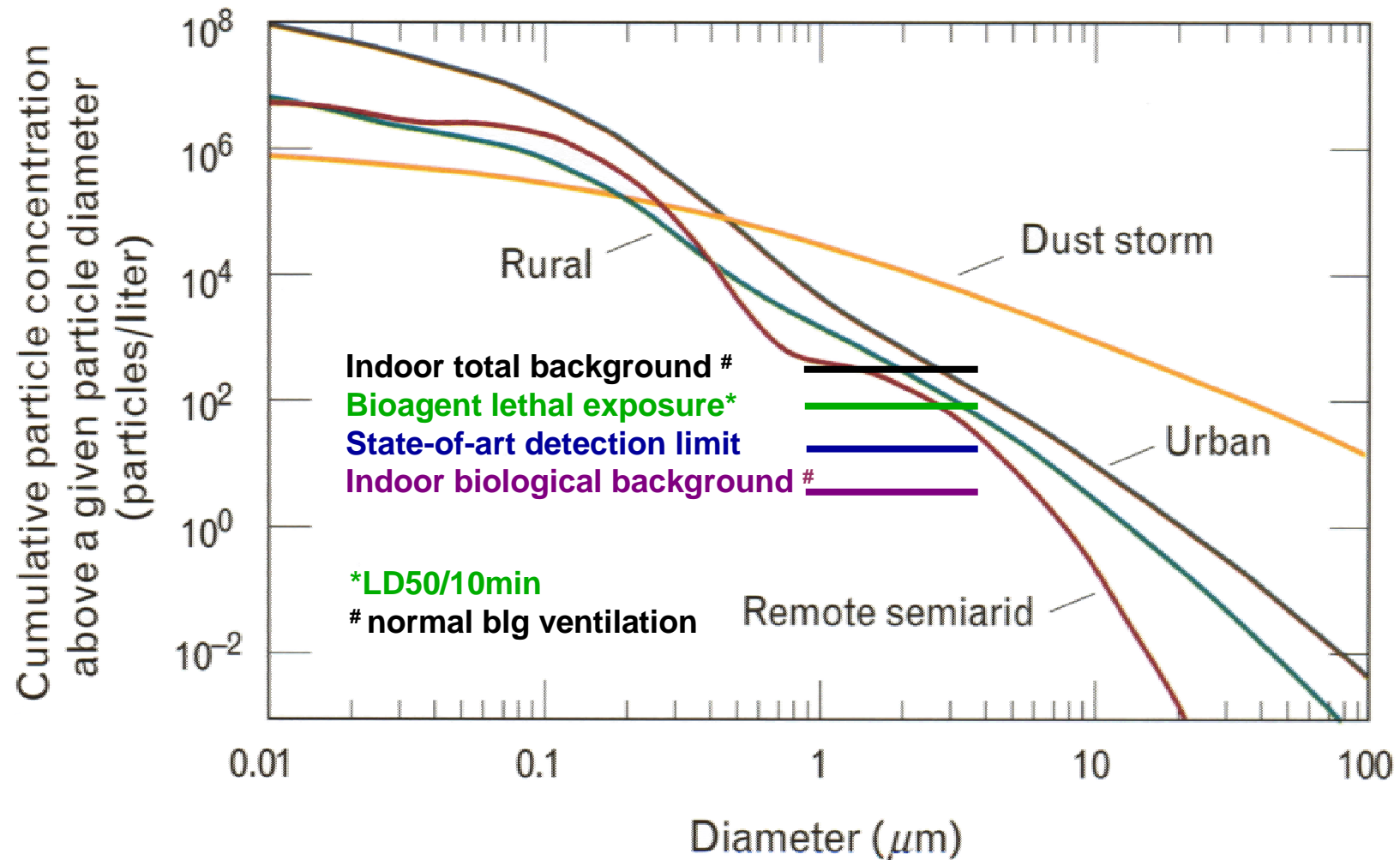


Sensor Architectures for Building Defense

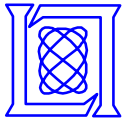




Atmospheric Aerosol Content

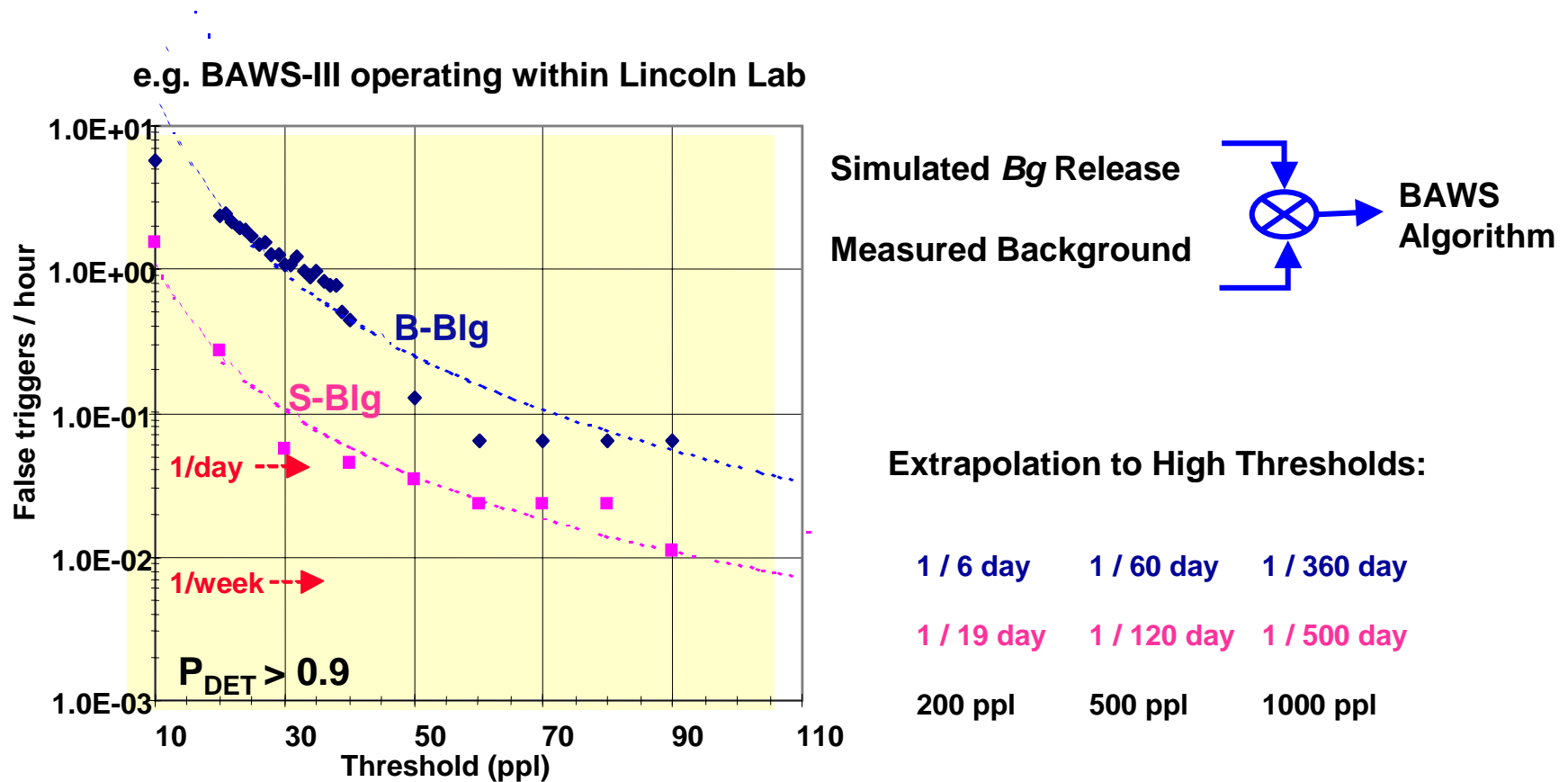


After R. Jaenicke in *Aerosol-Cloud-Climate Interactions*, P. Hobbs editor (1993).



False Trigger Rate

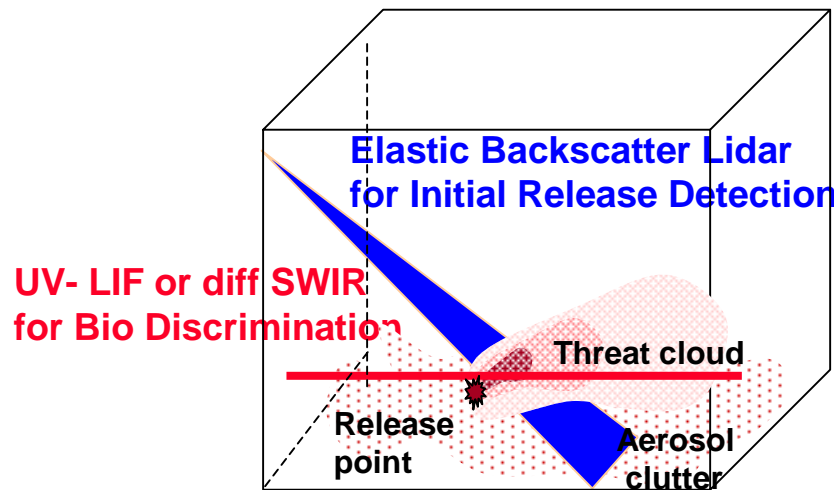
- Sensor will trigger less frequently when operated at higher threshold.





Indoor Standoff Aerosol Detection

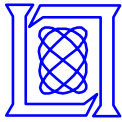
- Any point sensor is limited by aerosol transport in large open space.
- Need to detect the release promptly at a specific point
- Bio sensor concept:



Minimum for detecting 1000ppl threat

| | Dwell time | Range cell |
|-----------|---------------|---------------|
| Elastic | 0.1 sec | 1 m |
| UV LIF | 10 sec | 3 m |
| Diff SWIR | 10 sec | 2 m |

50m range, eyesafe laser; 100 lux lighting



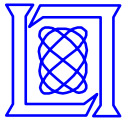
Outline

- Facilities and attack scenarios
- Sensing an attack
- Facility protection techniques



Facility Protection Measures

- **Physical security**
 - **Protect fresh air intakes (location, access, surveillance)**
 - **Personal screening (may be difficult in civil defense)**
- **Ventilation system protection**
 - **Passive air filtration**
 - › **Upgrade filters (best ASHRAE filters > 95%)**
 - › **Overhauling the system (HEPA / carbon)**
 - **Positive pressure to overcome infiltration**
 - **Sensor triggered airflow control**



Passive Air Filtration

- **In-line passive filtration is well established**
 - HEPA filters remove >99.97% suspended particles > 0.3 μm .
 - Activated carbon filters adsorb most chemical vapors
- **Substantial cost to overhaul existing ventilation system**
 - Purchase and replacement of filters
 - Increased blower motors for higher pressure drop
 - Reinforced ductwork
 - Very little infiltration is allowable (gasket seals, overpressure)
 - Increased energy costs
- **Research topics**
 - Low pressure drop filter structures
 - In-line sterilization (UV, radiation, thermal,...)



Facility Defense Effectiveness

Estimated exposure reduction
to external bio attack

- | | |
|--|----------|
| • “Unprotected” building | 1 |
| • Upgraded standard filters (or in-room HEPA) | 10-100 |
| • In-line HEPA filters | 100-1000 |
| • In-line HEPA filters with overpressure and triggered airflow control | > 1000 |



Summary

- **Most buildings with ventilation systems are vulnerable to aerosol attack via a number of scenarios.**
- **Without deployed sensors, an attack may go undetected resulting in higher exposure and lack of treatment to exposed occupants.**
- **There are some simple measures that can be used to increase situational awareness and provide limited protection.**
- **A substantial degree of protection can be achieved at substantial cost with sensor triggered airflow control and HEPA/carbon filters. In this case, sensors may be operated at higher thresholds.**

Aerosol Triggers

Thomas H. Jeys

New England Bioterrorism Preparedness Workshop

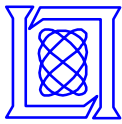
3-4 April 2002

MS-15436

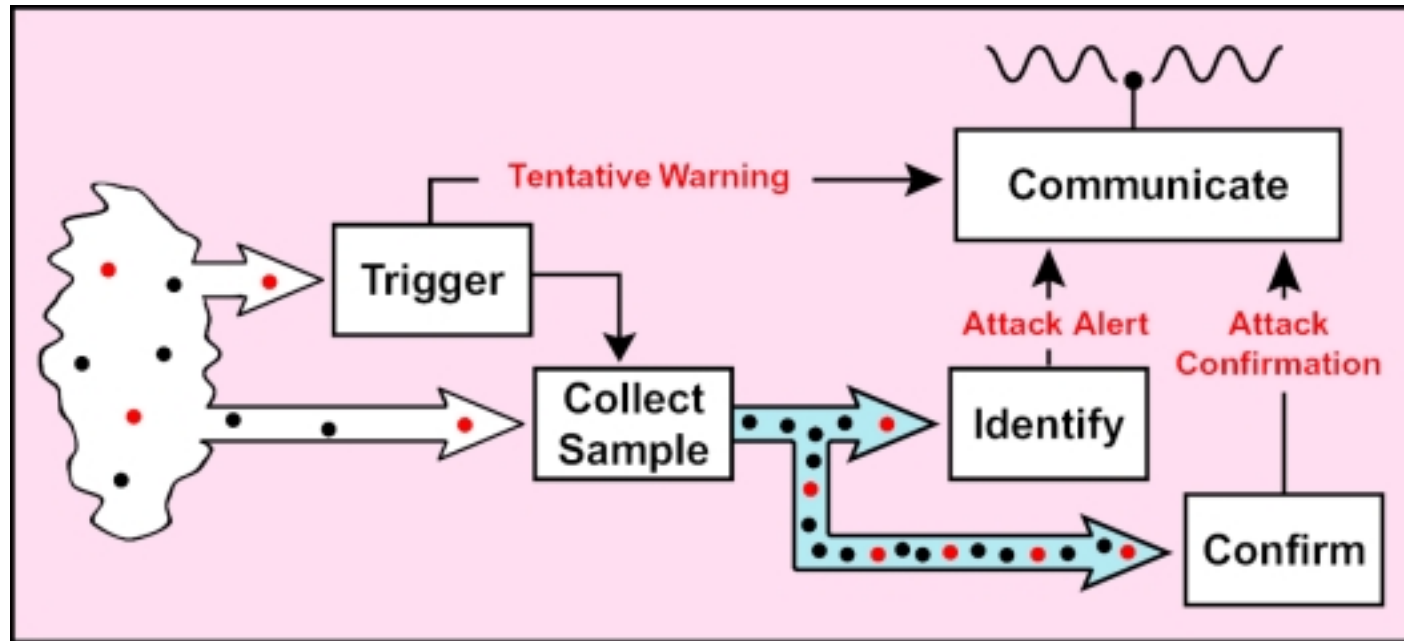
This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.

50
years

MIT
LINCOLN LAB



Biosensor Architecture



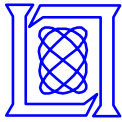
- **Trigger (< 60 s)**
 - Continuous operation
 - Alert of potential threat aerosol
- **Collector (5 min)**
 - Activated by trigger
 - Provide sample of aerosol particles

- **Identification (15 min)**
 - Preliminary identification of agent
- **Confirmation (4 – 24 hr)**
 - Final identification of agent
 - “Gold Standard” tests
 - Performed in laboratory (TAML)



Bio-Aerosol Triggers

- **Raw Particle Counters**
 - Small, low cost
 - Nondiscriminatory - very high false trigger rates
- **Fluorescent Particle Counters**
 - **Ultra Violet Aerodynamic Particle Sizer (UVAPS)**
Trigger for Biological Integrated Detection System (BIDS)
Manufactured by TSI Inc. (St. Paul, MN)
Fluorescence Aerodynamic Particle Sizer (FLAPS)
Different trigger algorithm than UVAPS
Trigger for Canadian Integrated Biological Agent Detection System (CIBADS)
 - **Biological Agent Detection Sensor (BAWS)**
Trigger for Joint Biological Point Detection System
Manufactured by Intellitec (Deland, FL)



Biological Agent Warning Sensor (BAWS)

- **Army Advanced Technology Demonstration**
 - Began BAWS development in 1996
- **Four design generations developed**
- **Extensively tested**
 - Performance
 - Environmental
- **Integrated into the Joint Biological Point Detection System**
 - Development transitioned to JBPDS in 1999.

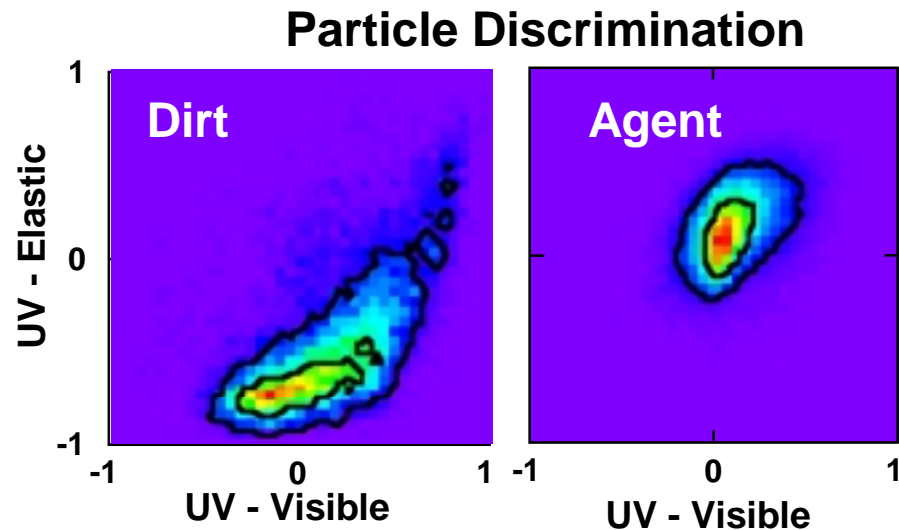
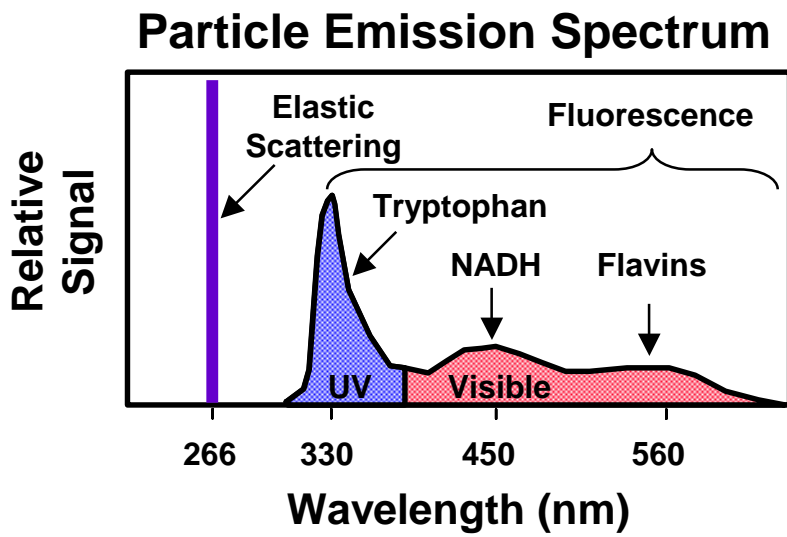
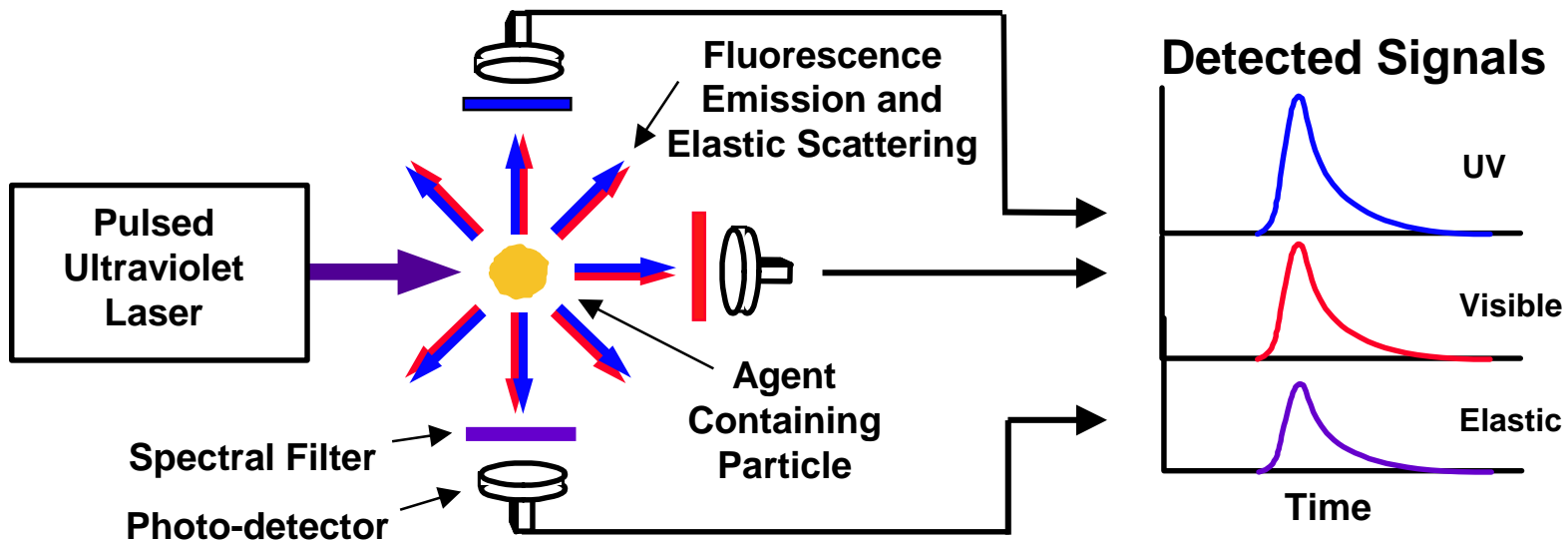
BAWS III



| | |
|--------|---------------------|
| Size | 0.8 ft ³ |
| Weight | 19 lbs |
| Power | 35 W |



BAWS Concept





Joint Biological Point Detection System



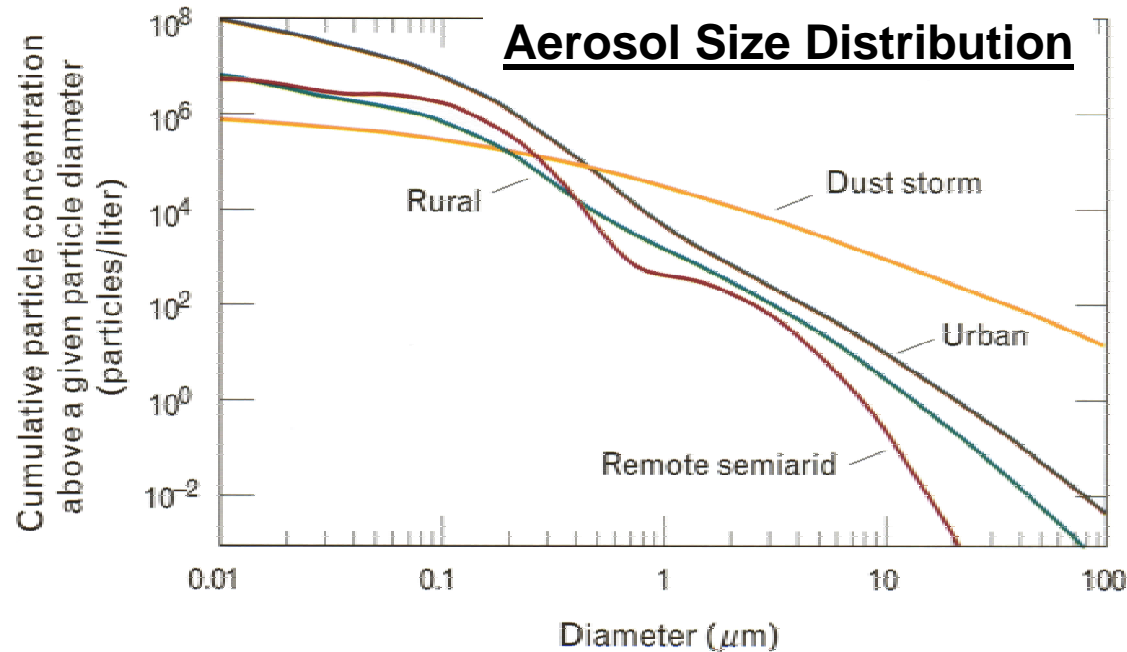
- Automated suite of sensors for detection and identification of biological attacks
 - Trigger – BAWS
 - Collector – Wetted Wall Cyclone
 - Identifier – Immunoassay
 - Confirmatory Samples



BAWS



The Atmospheric Aerosol Composition



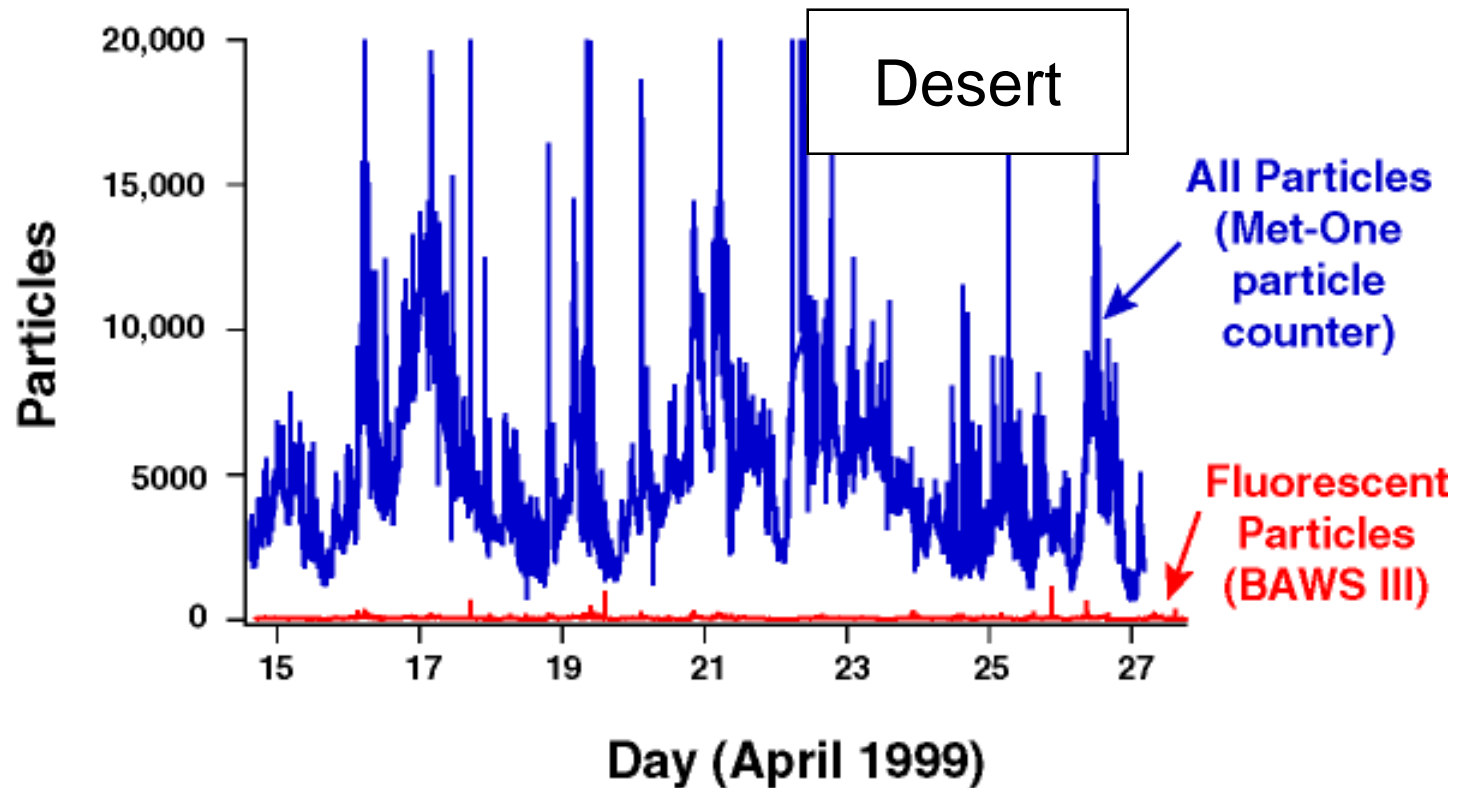
*After R. Jaenicke in Aerosol-Cloud-Climate Interactions, P. Hobbs editor (1993).

Composition of Coarse (>1 micron) Aerosol

| Organic Aerosols | Particles per Liter | | Inorganic Aerosols |
|-----------------------|---------------------|--------------|--------------------------|
| Man Made | 0 – 2000 | 100 – 10,000 | Clays, Sands, Composites |
| Fungi | 0 – 100 | | |
| Bacteria (culturable) | 0 – 1 | | |
| Pollen | 0 – 1 | | |



Total vs. Fluorescent Particles



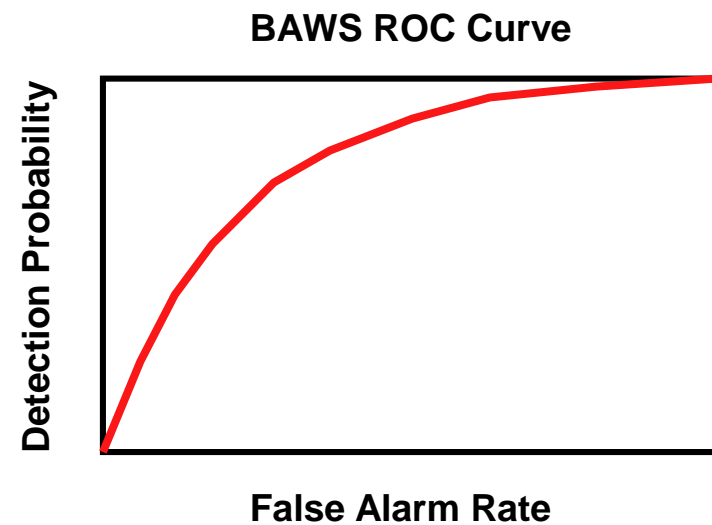
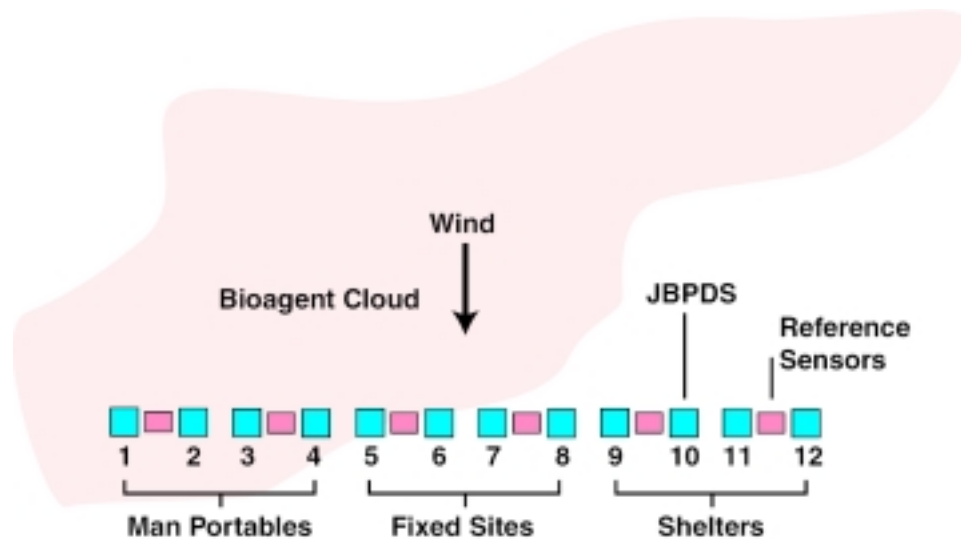
- Most sand particles do not fluoresce and are “invisible” to BAWS



Field Trials

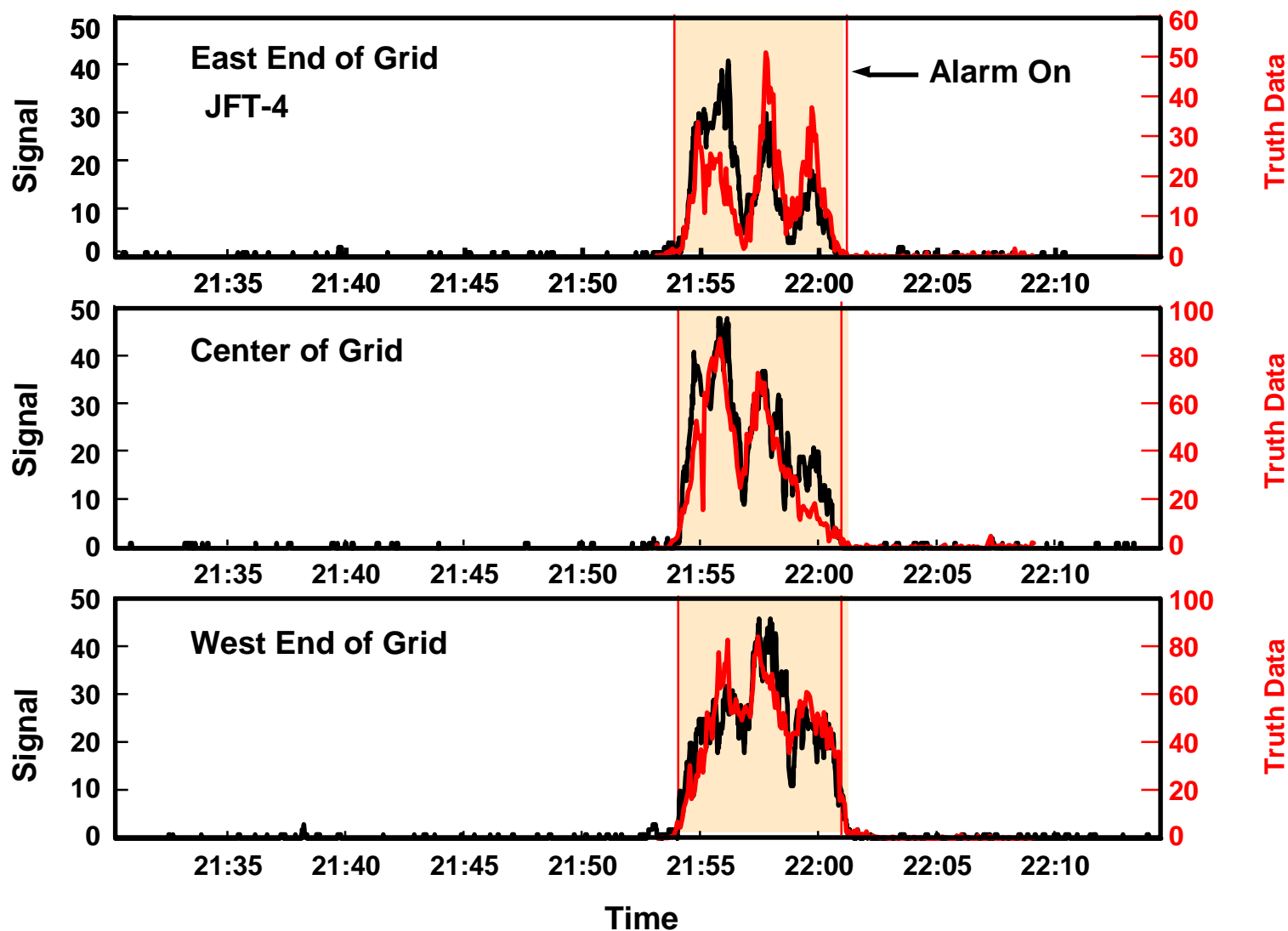


27Oct99 - 9Nov99
BAWS/JBPDS Mini Field Trials





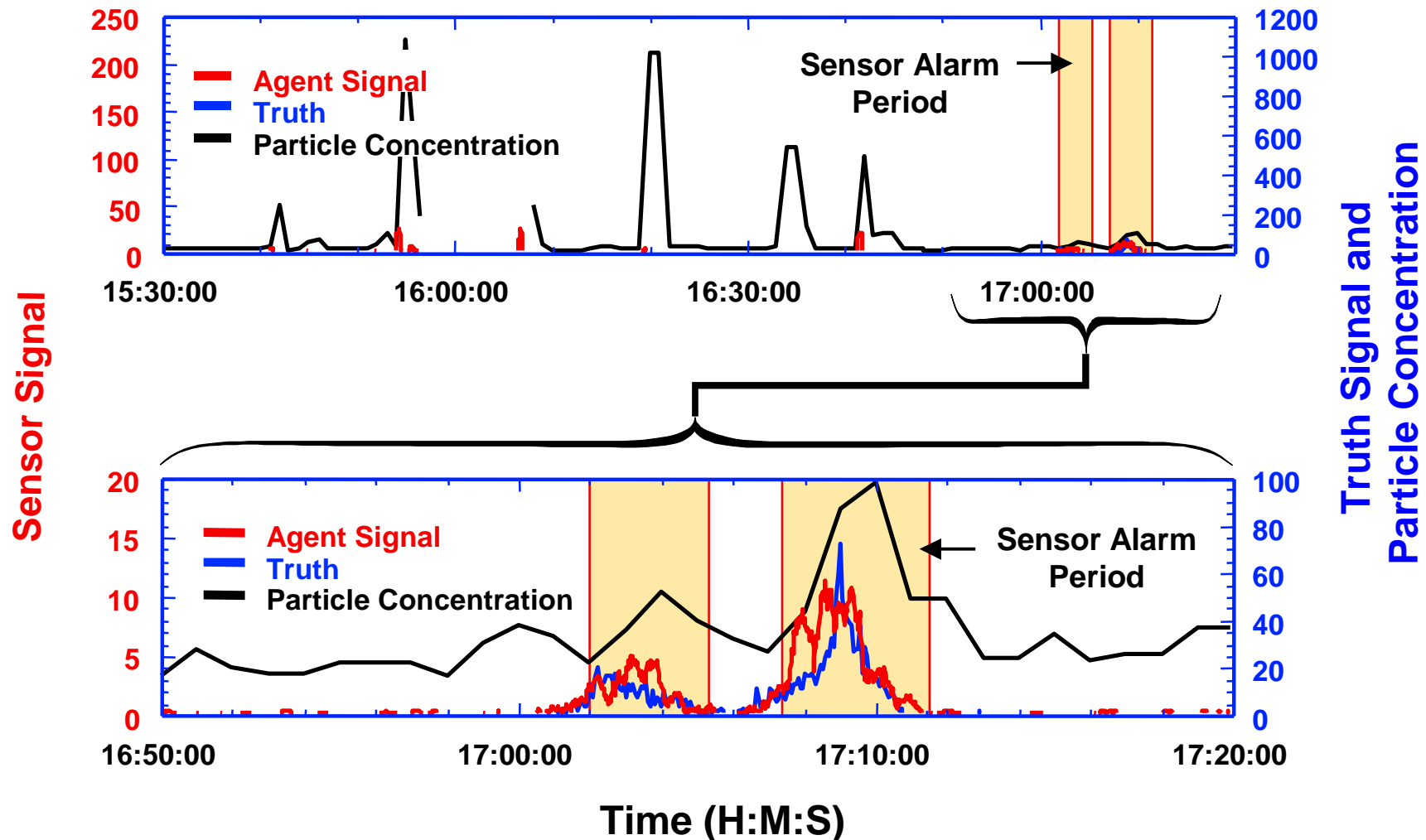
Response of BAWS Array to Agent Aerosol

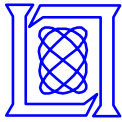




Response of BAWS to Interferent and Agent Aerosol

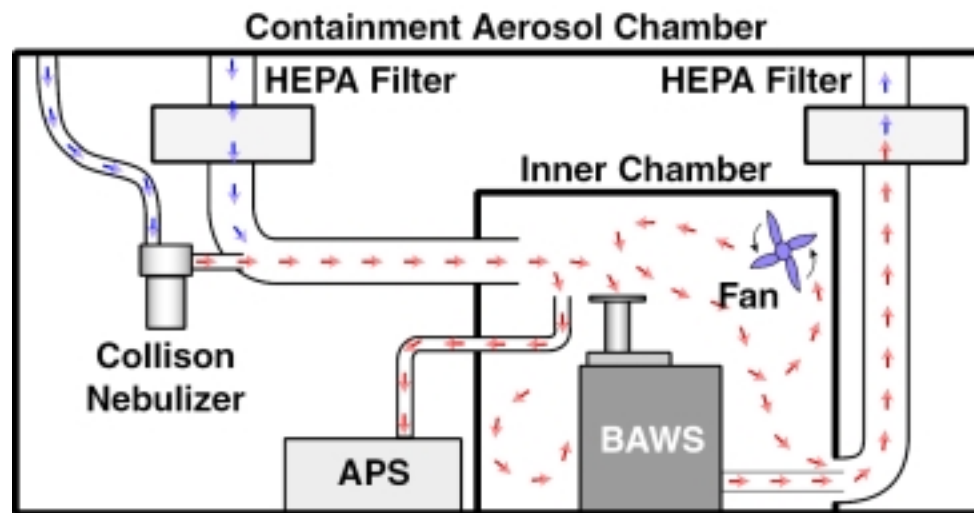
Sensor at East End of Grid





Live Agent Tests of BAWS

- **Comparison of BAWS response to real agents and simulant agents**
 - Simulant Agents; BG, *Erwinia herbicola*, Ovalbumin, MS2
 - Three Real agents



- **Results: BAWS detects live agents as well as, or better than, simulant agents**
 - Equivalent sensitivity
 - Equivalent discrimination

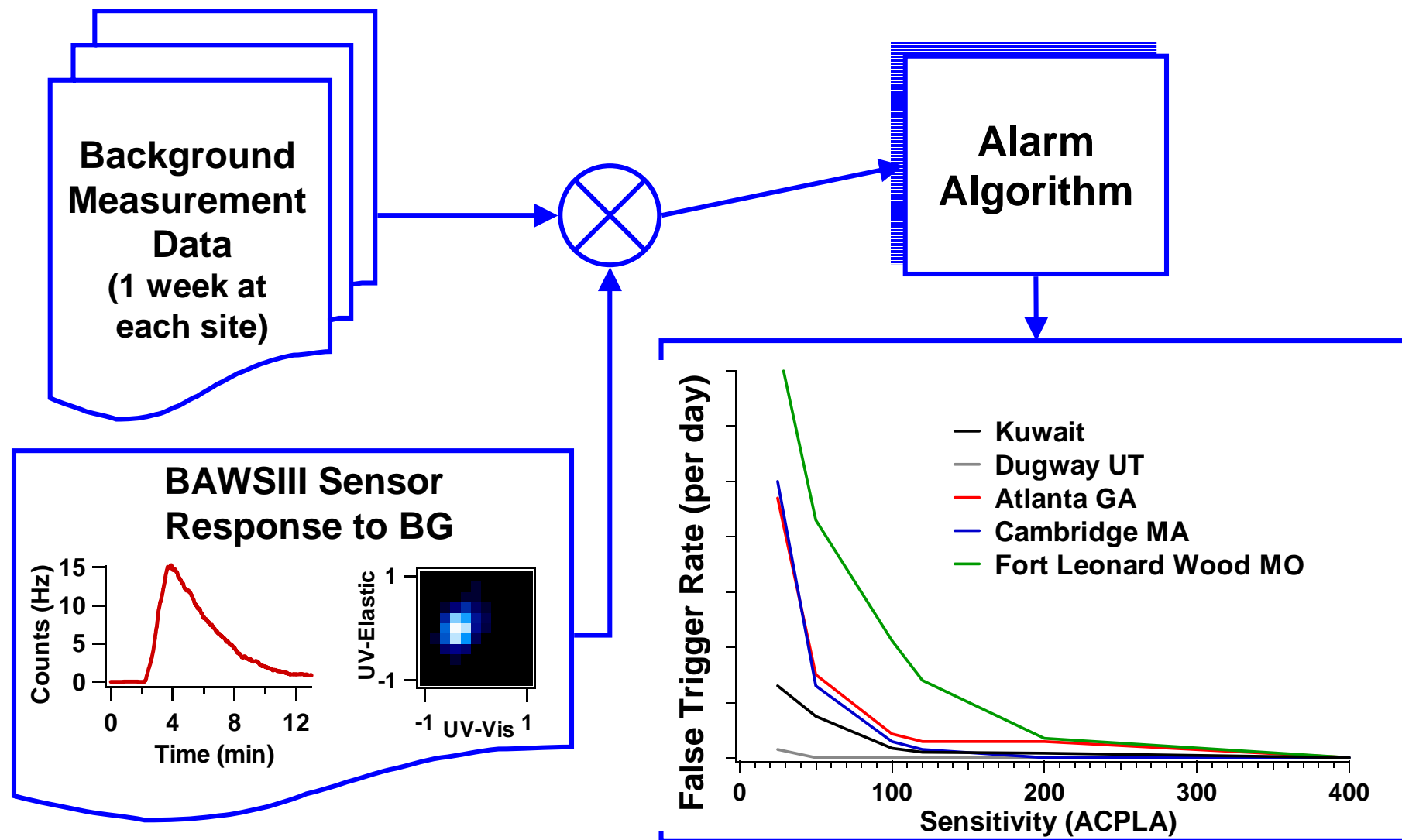


BAWS Performance Testing

- **Joint Field Trials**
 - JFT 3, Dugway Fall '96
 - JFT 4, Dugway Fall '97
 - JFT 4.5, Dugway Spring '98
 - JFT 6, DRES Canada Fall '00
- **Army ATD Field Trials** Spring '99
- **Joint Biological Point Detection System Field Trials**
 - Mini Field Trials Fall '99
 - Gamma-Killed Bio-Agents Spring '99
 - PPQT Spring '00
 - Live Agents Summer '00
 - Porton Down, UK Fall '00
 - Ambient Breeze Tunnel, Battelle Spring '01
 - Operational Assessment 2 Fall '01
- **Background Measurements**
 - USA tour '98 – '99
 - Kuwait Spring '99
 - Altitude study Fall '00
 - Salt Lake City Spring '01
 - Hawaii Summer '01



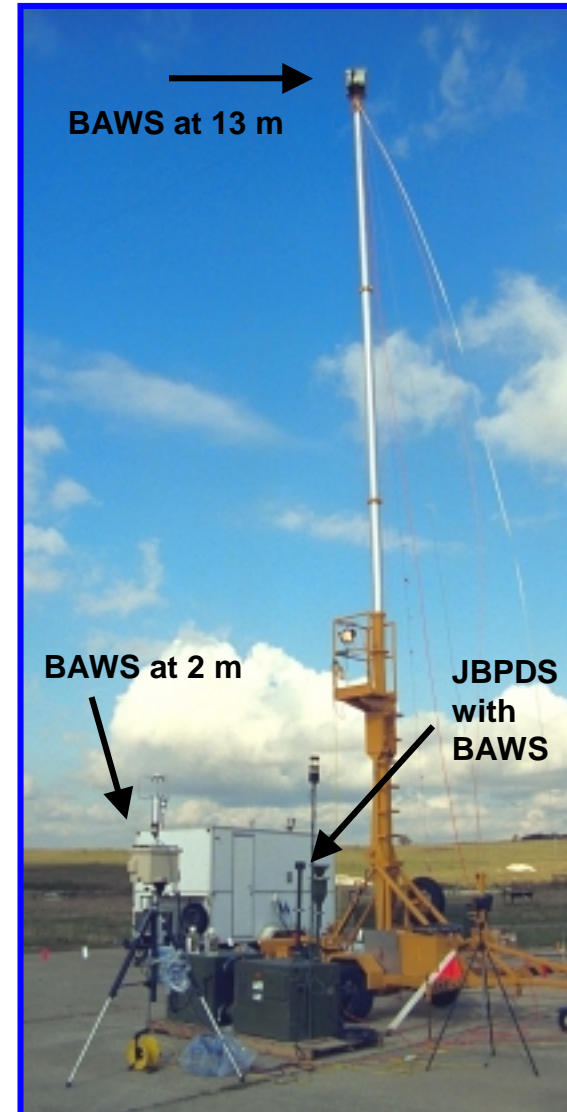
Simulation of BAWS Response to Agent Attacks in Different Environments





Detector Position vs. False Trigger Rate

- **England (Sep '00)**
 - One week of measurements
 - 21 agent simulant challenges
 - 8 interferent challenges
- **Sensor Performance vs. sensor height**
 - BAWS at 2-m and 13-m height
 - Ten times fewer false triggers at 13-m height





Summary

- **BAWS developed for early warning of a biological agent attack**
 - continuously operating point detector
 - small size, low weight, low power consumption
- **Generic detection (not identification) of threat aerosol**
 - Individual detection of aerosol particles
 - Discrimination of threat particles from non-threat particles
 - Sensitive, low false alarm rate, fast response
- **Subjected to extensive testing**
 - Performance
 - environmental
- **BAWS integrated into JBPDS**



Emerging Technologies in Sample Analysis

4 April 2002

New England Bioterrorism Preparedness Workshop
MIT Lincoln Laboratory

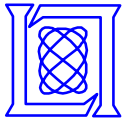
Bernadette Johnson
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bernadette@ll.mit.edu

MS-15453

This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.

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years





Outline

- **Current techniques in sample analysis**
 - Clinical (subject of yesterday's talk)
 - Environmental
- **Challenges associated with environmental sampling**
- **Examples of technologies in use and in development**

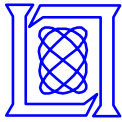


CDC's Sample Analysis Guidelines

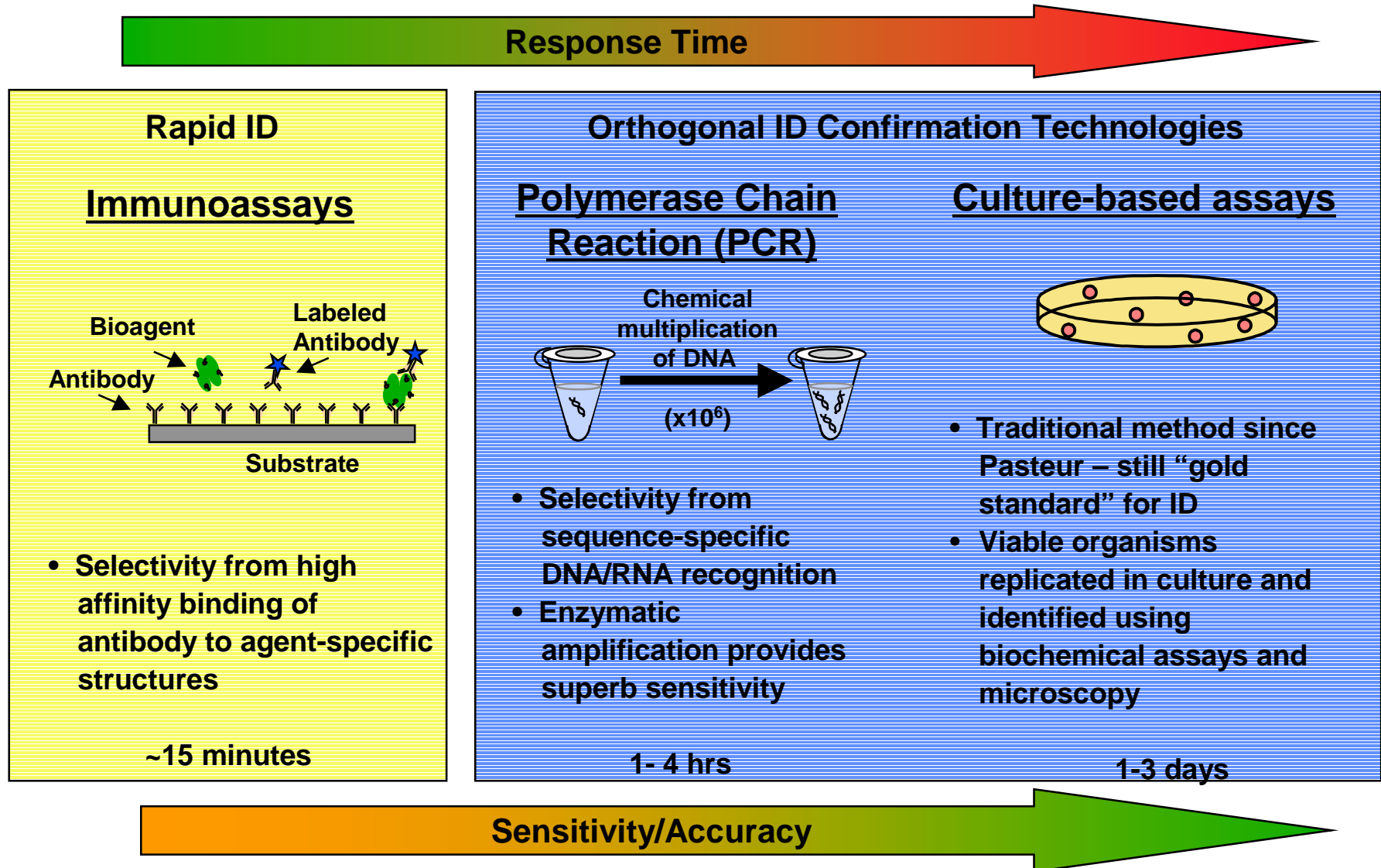
(example: *B. Anthracis*)

- **Persons suspected of exposure/infection**
 - Cultures of blood and spinal fluid
 - Cultures of tissues or fluids from affected areas
 - Microscopic examination
 - PCR
 - Nasal swab (occasionally for exposure, but not for diagnosis)
 - Antibody testing (exposure, not validated for diagnosis)

- **Environmental contamination**
 - Cultures of air samples, surface swabs, suspicious powders
 - Microscopic examination of suspect material
 - Evaluation of growth properties of suspect agent
 - PCR
 - DFA (direct fluorescent assay) to detect key bacterial proteins
 - Specialized tests, such as immunoassays (SMART)



How Do These Techniques Compare?





Examples of In-use and **Developmental** Immunoassay Devices



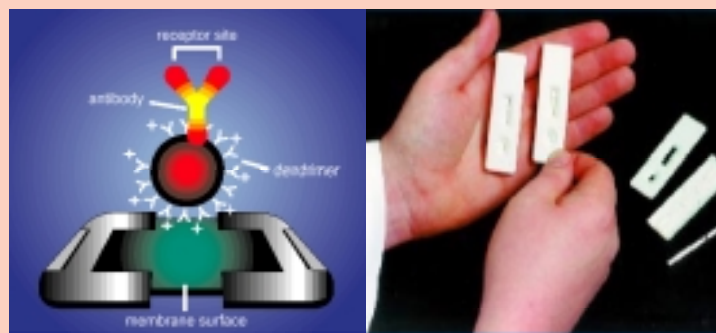
← 4.5 in →



**Ticket cartridges and reader
for lateral-flow immunoassay
in Joint Biological Point Detection System
(JBPDS)**

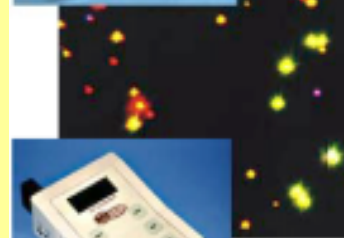


**Response Equipment Co.
Bio-HAZ Biodetector**



Dendrimer-Based Alert Ticket (ARL)

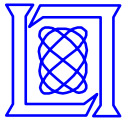
Disposable Test Strip



Prototype Design



**Upconverting
Phosphors (SRI)**

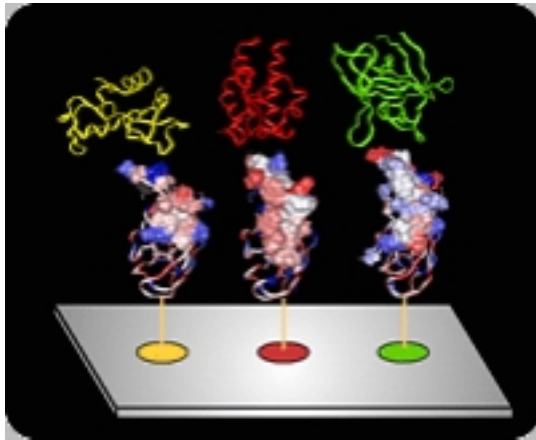


Features of Immunoassay Analysis

- Can be used on environmental samples with little or no preparation
- Readout is fast (~ 15 minutes) and simple (colorimetric or fluorimetric)
- Sensitivity modest (~10,000 - 100,000 particles)
 - Depends on antibody-antigen binding affinity and readout scheme
- Specificity reasonably good
 - Depends on antibody construct and antigen specificity
- Current IAs are not multiplexed; development of protein microarrays may lead to sensitive, multi-assay analysis tools



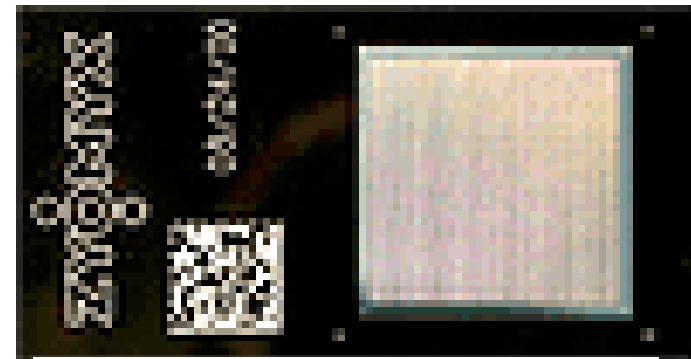
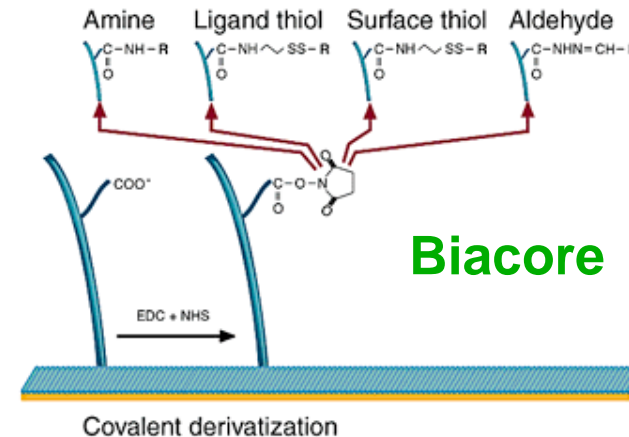
Examples of Existing Protein Microarrays



Phylos (2000 element)



Ciphergen (multiple classes of proteins)



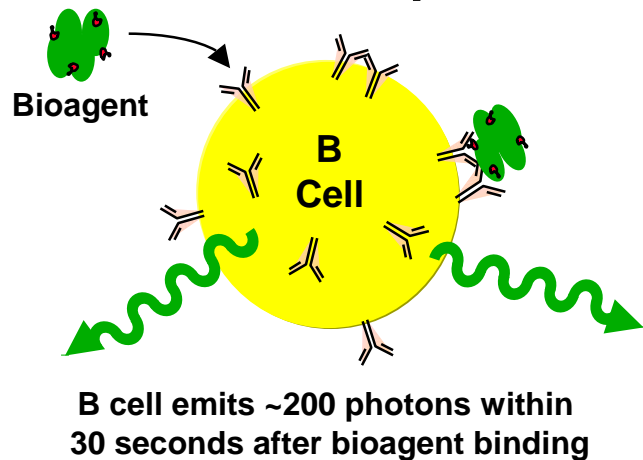
Zyomyx (10,000/cm²)

- Protein microarray technology development driven by drug screening and disease-marker investigations
 - Diagnostics (clinical and environmental) still developmental

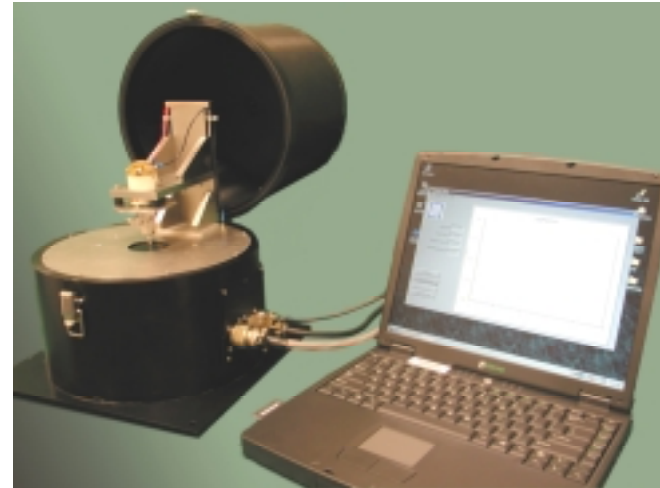


Developmental Antibody-Based Sensor: CANARY

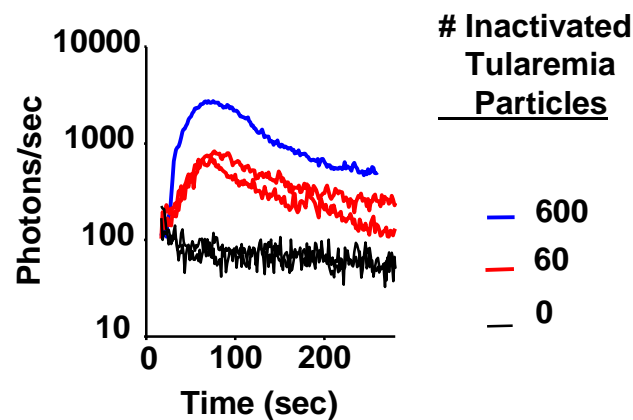
Concept



Prototype microcentrifuge device



Tests Against Killed Tularemia (Collab. with NMRC)



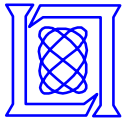
Status of B-Cell Lines

Complete

FMDV
VEE
Vibrio cholera
Orthopox viruses
Yersinia pestis
Brucella spp
Francisella tularensis

In development

Coxiella burnetti
Bacillus anthracis
E. coli O157:H7



PCR-Based Analysis Tools

- **Systems being developed (and deployed) that provide agent ID within 30 minutes of introduction of prepared sample**

Semi-automated field-portable
PCR devices



RAPID - Idaho Technologies



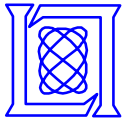
***SmartCycler
XC System - Cepheid***

Example of handheld PCR
device

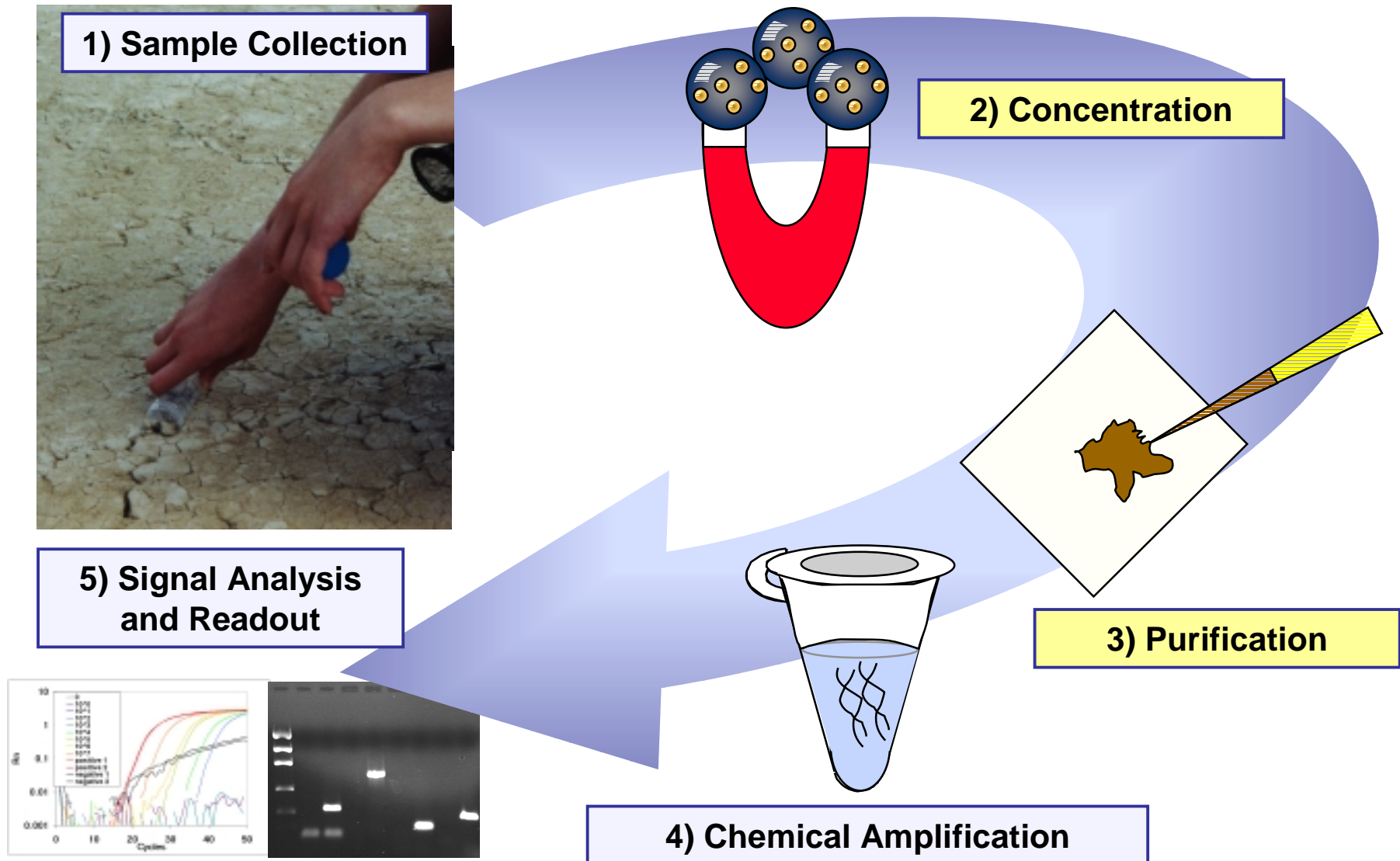


***HANAA - Handheld Nucleic
Acid Analyzer, developed by
LLNL, Cepheid, and ETG, Inc.***

- **Challenge remains in automating sample preparation and analysis**
 - Pathogen cells or spores must be ruptured to liberate the DNA/RNA
 - DNA/RNA must be separated from protein debris/environmental impurities

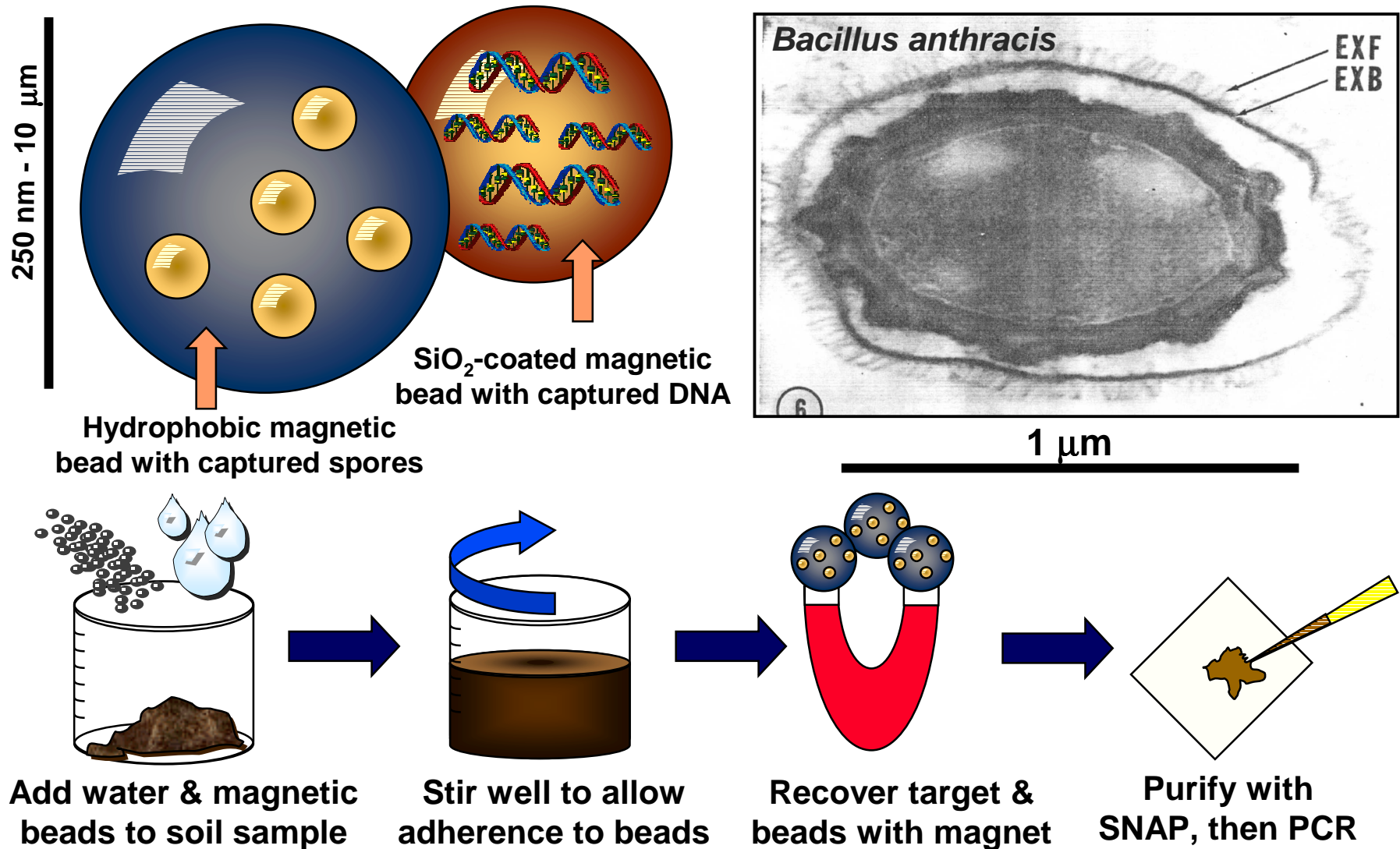


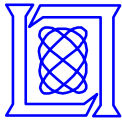
Overview of Sample Preparation





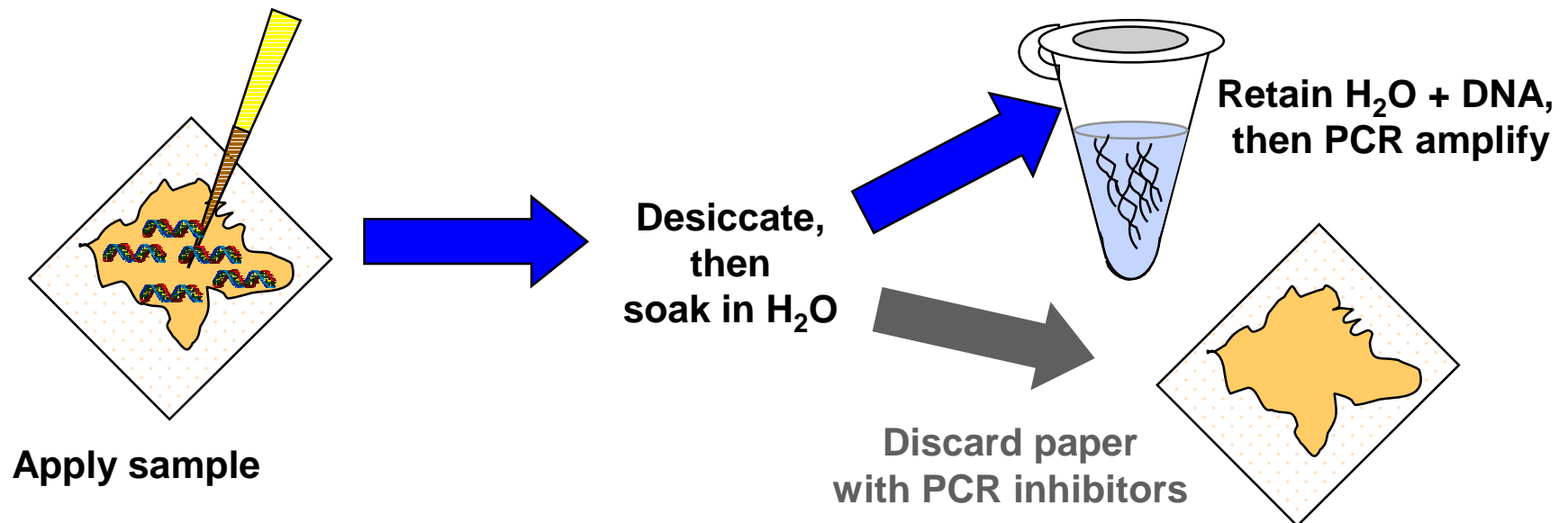
Target Concentration: Affinity Magnet Protocol





DNA Purification: Simple Nucleic Acid Prep (SNAP)

- Chemically treated paper is the key component of SNAP
- Lyses cells, binds PCR-assay inhibitors, and purifies DNA
- **Advantages:**
 - Fast and easy (1/5th the time of other published protocols)
 - Water is only added reagent (no phenol, chloroform, or alcohol)
 - Lightweight, compact, enables archiving
 - On-site fixation: preserves DNA & kills pathogenic organisms

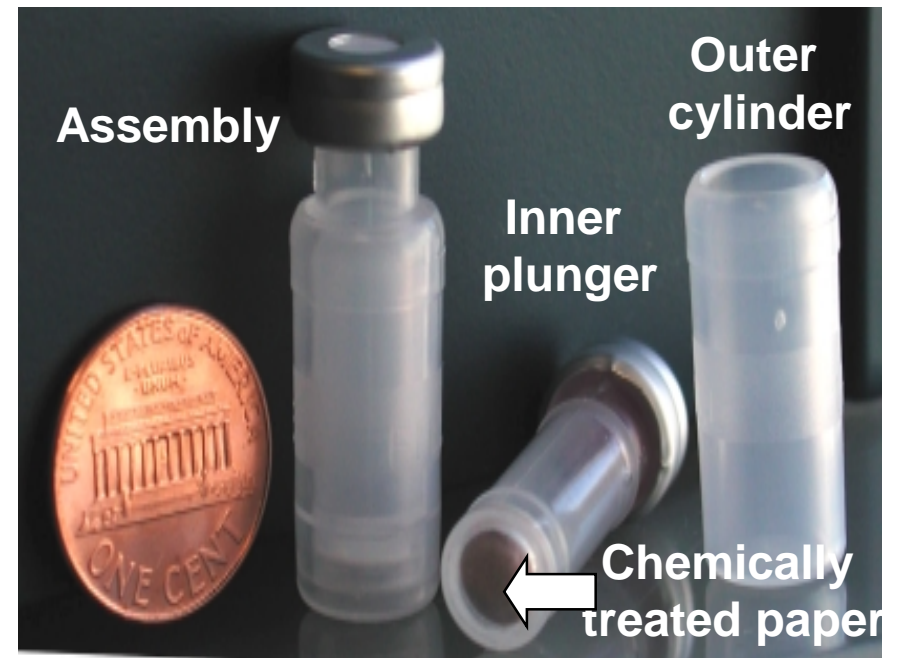




Lincoln Interim Nucleic-acid Kit (LINK)

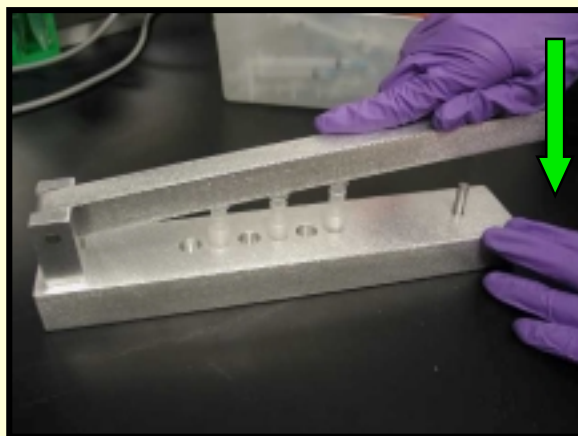
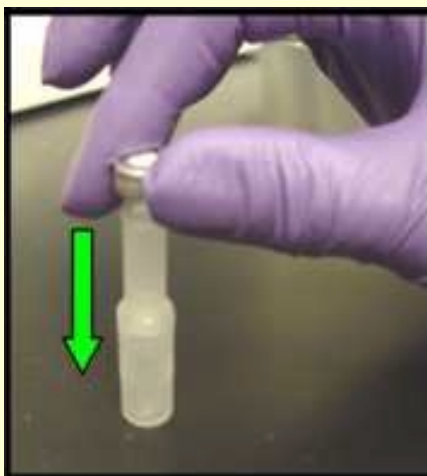
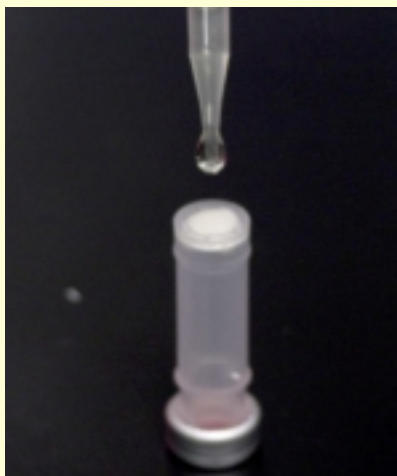
(Developed in response to October 2001 events)

- **LINK as a solution:**
 - Incorporates SNAP paper but in a more user-friendly format
 - Faster processing than basic SNAP
 - Easier to sample, handle, and process
 - Enables on-site fixation
 - Outside can be decontaminated
 - 6 minute processing time
 - Single-step processing
 - Results equal to or better than basic SNAP





How to Use LINK



1) Apply sample
Sit for 5 minutes

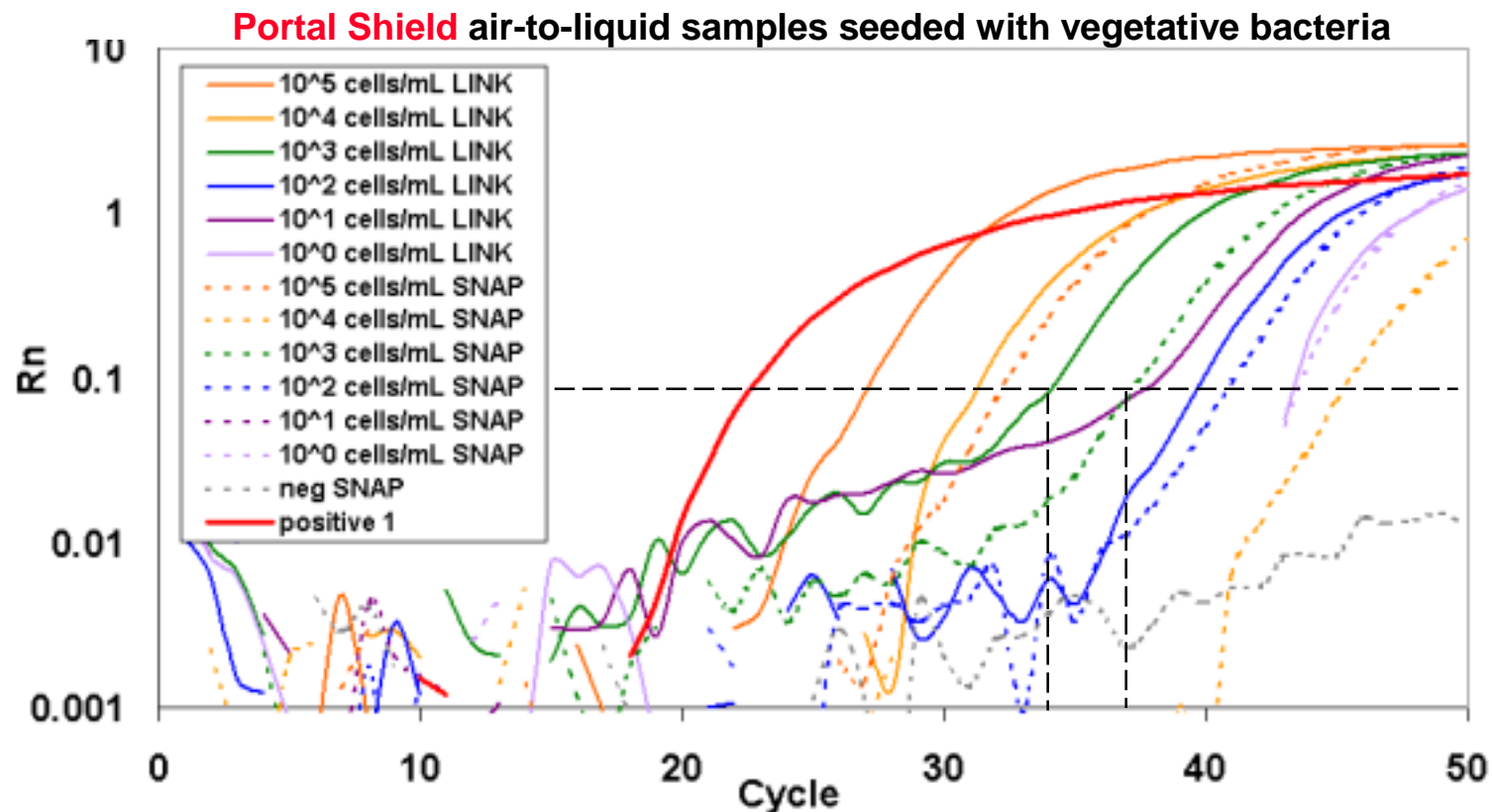
2) Process in **one step**

3) Remove DNA
Total time ~6 minutes!



LINK Cartridge Works with Varied Samples

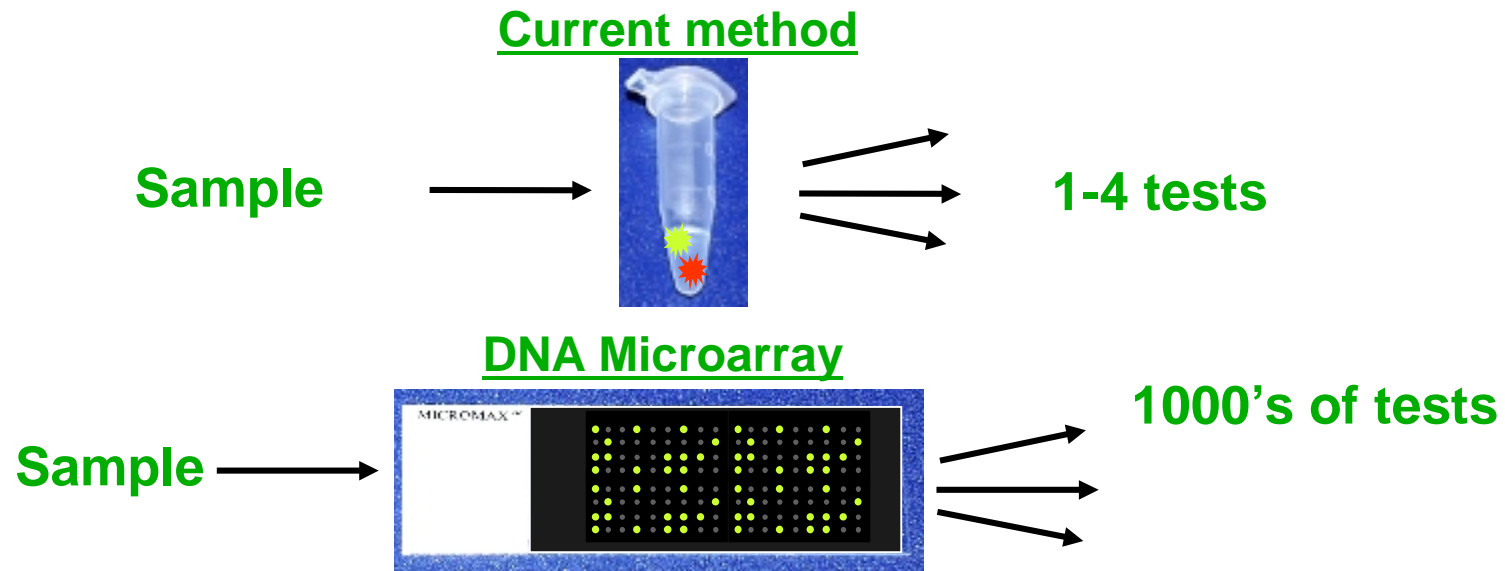
- LINK detection from:
 - **Portal Shield** air-to-liquid samples seeded with vegetative bacteria
 - Untreated domestic sewage (Boston) seeded with vegetative bacteria
 - Paper, envelopes, skin seeded with bacterial spores
 - Air impaction with dry bacterial spores





What About DNA Microarrays?

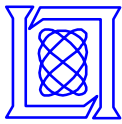
- DNA Microarray: Any 2D or 3D substrate having many ($\sim 10^2$ - 10^5) different nucleic-acid capture sites (probes)
- Can identify both strain and drug resistance of pathogens
- Can offer highly multiplexed assay capability



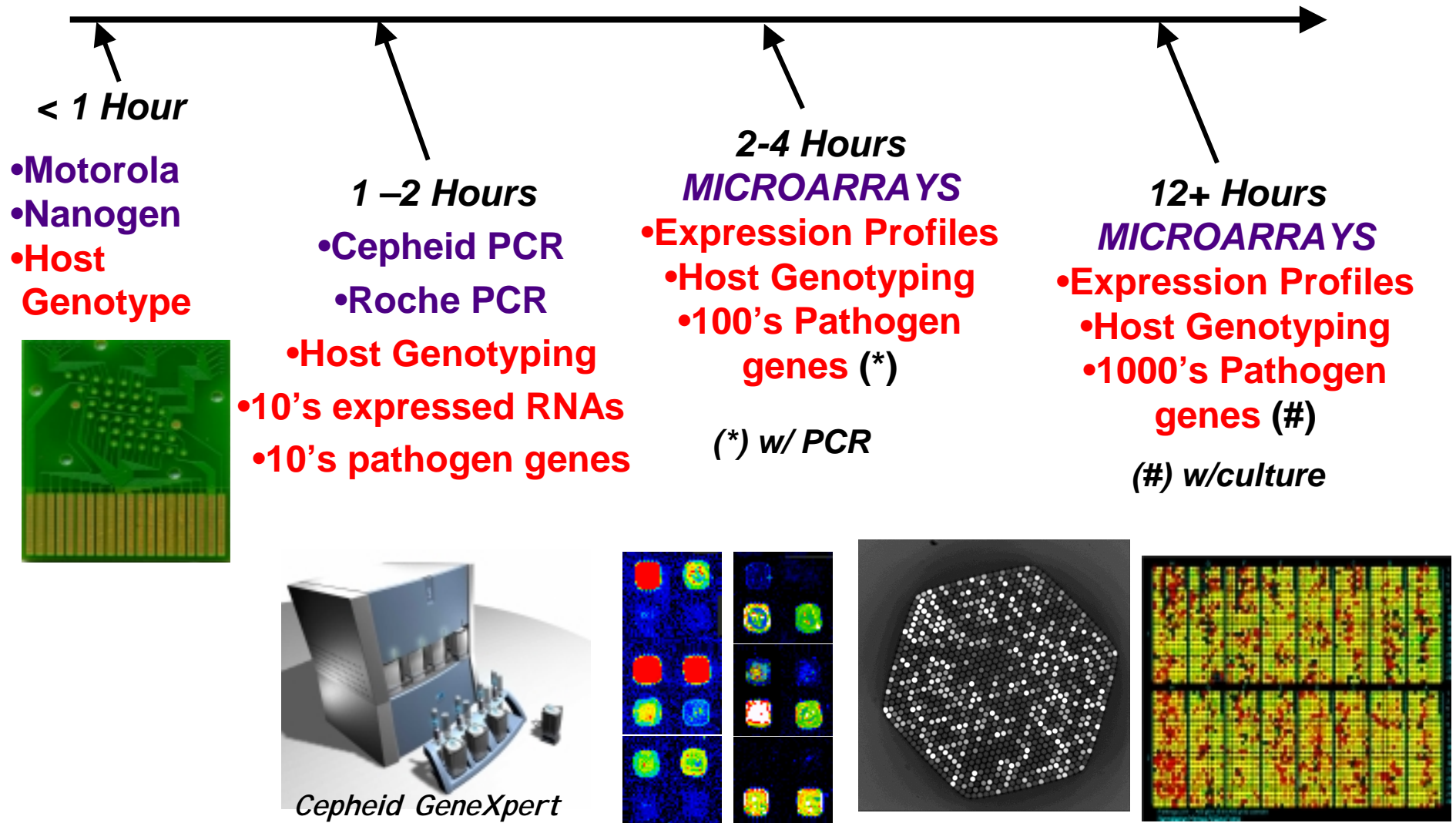


Pathogen Identification via DNA Microarray

- Detect small amounts (<100 copies per ml) of pathogen-specific nucleic acids in environmental sample
- Arrays might provide log orders more information than current PCR-based approaches (e.g. TaqMan)
- Challenges for diagnostic applications:
 - Never demonstrated for environmental (or clinical) samples
 - Amplification may be necessary before micro-array assay
 - Sample preparation required (as in PCR techniques)




Assay Times for Current and Emerging PCR/DNA Systems





Summary

- **Environmental sample analysis parallels methodology developed for clinical sampling**
 - Immunoassays for rapid estimate of exposure (not yet CDC authorized)
 - PCR techniques being deployed in some laboratories to provide strain specificity and drug resistance
 - Culture still used to provide “gold standard” for pathogen ID
- **New technology developments could greatly increase the speed, sensitivity, and multiplicity of environmental assays**
 - Protein microarrays could offer highly multiplexed, rapid ID capability on collected samples
 - DNA microarrays could offer hundreds to thousands of pathogen tests on single-chip format, provided sample preparation can be made compatible



Improving Public Health Measures: Advances in Risk Analysis



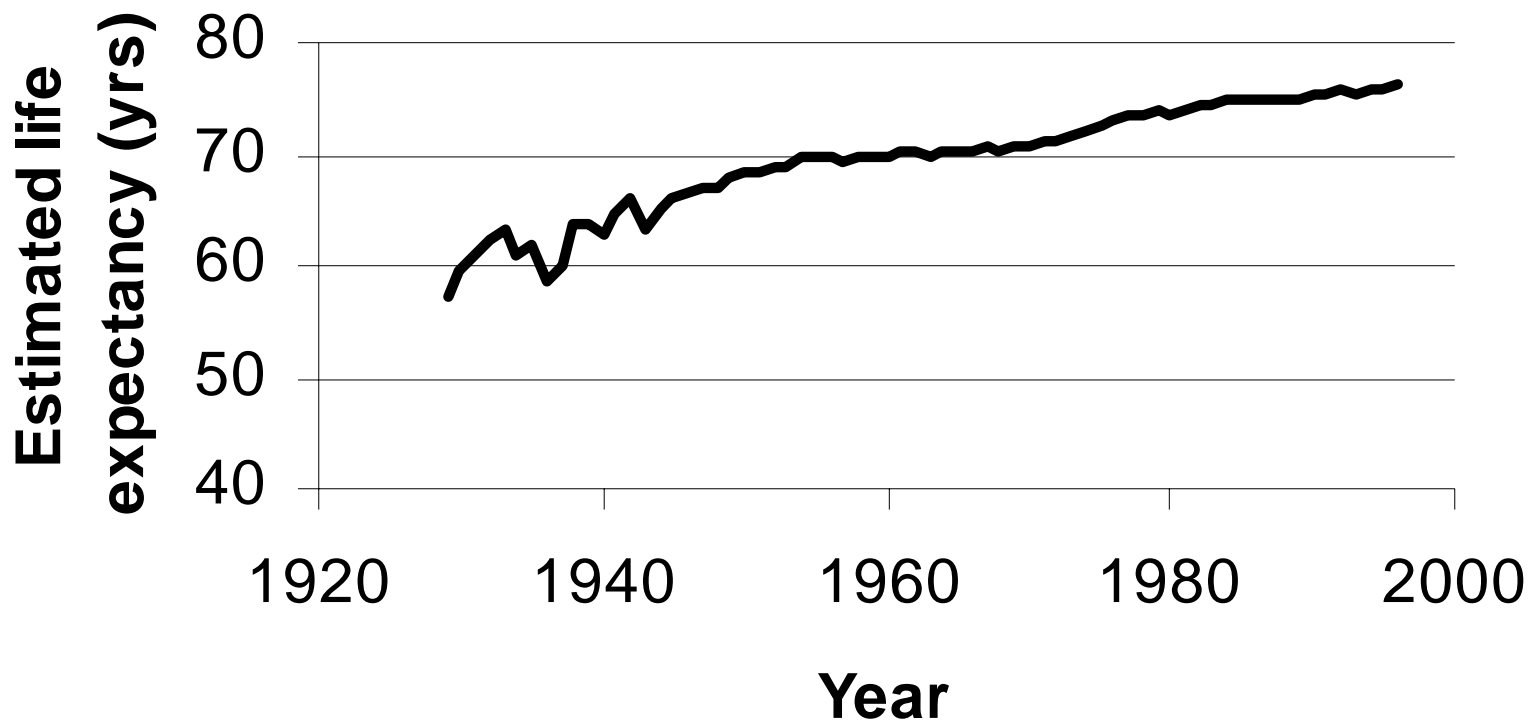
Kimberly M. Thompson, Sc.D.
Harvard School of Public Health
Boston, Massachusetts

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Where are we?

- The Age of Ignorance (no understanding of science, no control, all R no B)
 - Cotton Mather on colonial times: “A dead child was a sight no more surprising than a broken pitcher”
- The Age of Discovery (revolution in science, ability to understand and control disease, take R to get B)
- The Age of Miracles (idea of the magic pill or magic bullet, science can cure any problem, pursue B with abandon)
- The Age of Risk Management (science is critical, but we have to make good choices to avoid overkill, balancing R and B)

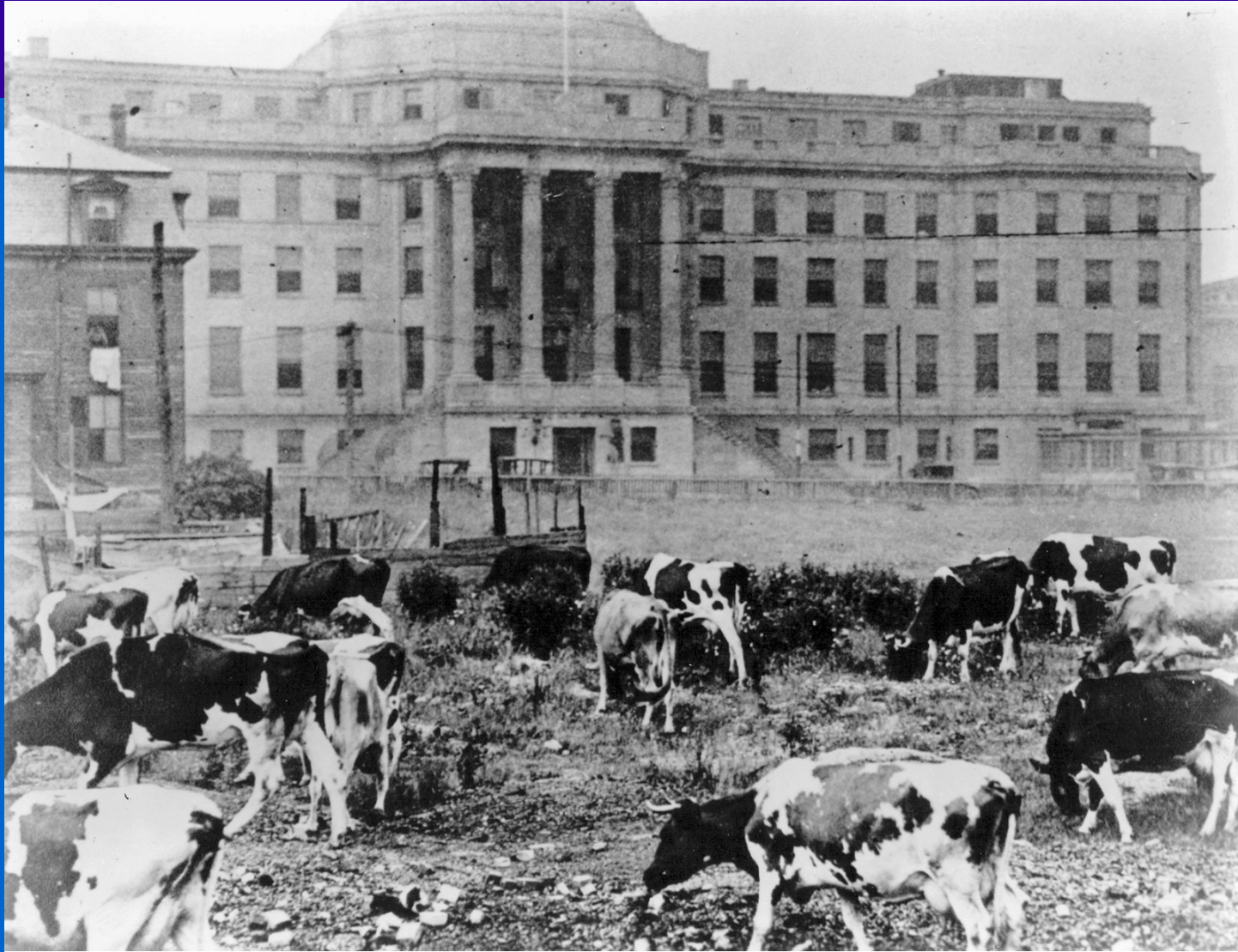
What does this mean for public health? Longer lives...



• • Great progress – a few examples

- Diagnosis of disease based on gross physical characteristics --> laboratory analyses of body fluids and genetic testing and interventions that save lives
- Sulfanilamide --> numerous antibiotics
- Focus on feeding and milk composition for infants --> pasteurization, refrigeration, infant formulas, dehydration treatments, and improvements in medical care

• Hunnewell Building, Circa 1914



Photograph courtesy of Children's Hospital Archives, Boston, MA.

© 2002, Kimberly M. Thompson, Sc.D.

Great progress

- The iron lung and deformities associated with polio --> immunizations for polio and many other diseases and eradication of small pox



Photograph courtesy of Children's Hospital Archives, Boston, MA.

© 2002, Kimberly M. Thompson, Sc.D.

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Public health improvements

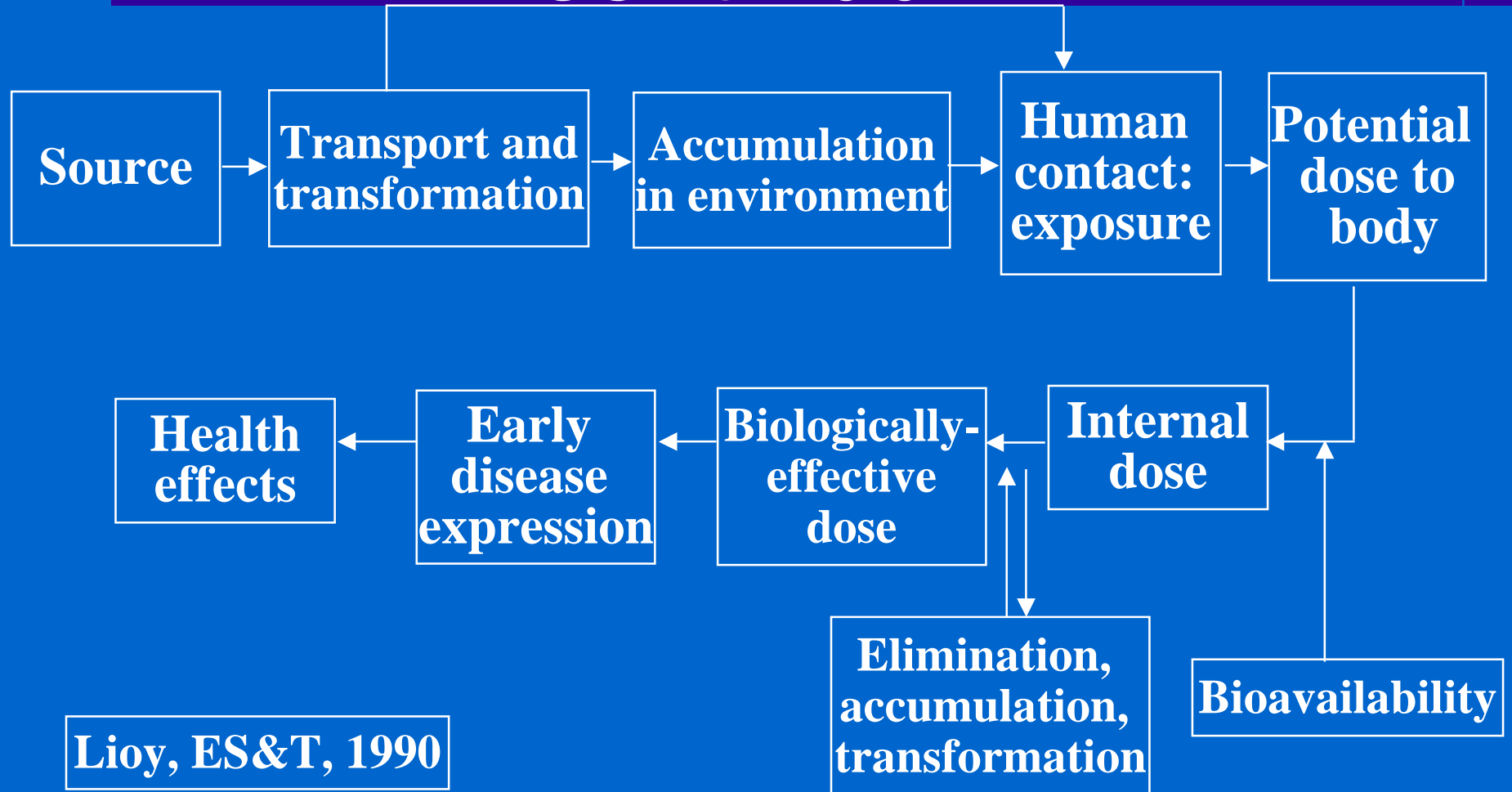
- Are we winning the war with germs?
 - Certainly doing better with respect to health outcomes (e.g., saving lives once lost to some infections, and reducing the severity and spread of infections)
 - Public perception now that infectious disease is not as much of a problem (immunization)
- Wait
 - BIG issues remain with antibiotic resistance/“Superbugs”/ new diseases
 - Prevailing assumption that releases of organisms would be unintentional (i.e., we’re fighting nature)
 - Infectious disease still a leading cause of death

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Context

- Given the background of ID, what does BT preparedness look like and how does it fit in with basic public health?
- What tools can help us understand the risks and measure the impacts of interventions?
- How will we know that a BT preparedness program works?
- What decisions get made about characterizing the different agents?

Human health risk continuum



The need for risk and decision analyses

- Risk analysis and decision analysis are used to integrate information and sift it down into a usable form
- Used support many actions:
 - Initiating regulatory activity or treatments
 - Setting protective standards
 - Selecting products, technologies, or substances
 - Siting hazardous facilities, isolation choices
 - Cleaning up or control of contaminated areas
 - Initiating research and establishing priorities
 - Others....
- Key component of decision (but not only)



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Decision tools

- Risk analysis
- Benefit-cost analysis
- Cost-effectiveness analysis
- Decision analysis
- Comparative risk analysis

All share common elements to some degree, but differences do matter

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Variability vs. uncertainty

- **Variability** - heterogeneity or diversity in a well-characterized population which is usually not reducible through further measurement or study
- **Uncertainty** - ignorance about a poorly characterized phenomenon that is sometimes reducible through further measurement or study
- **Variability and Uncertainty = f(decision context)**
 - **NRC (1994):** “Uncertainty forces decision makers to judge how probable it is that risks will be overestimated or underestimated for every member of the exposed population, whereas variability forces them to cope with the certainty that different individuals will be subjected to Techniques exist to maintain these separately
 - **risks both above and below any reference point one chooses.”**

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Risk estimates do matter

- Example 1 – uncertainty about the effectiveness of airbags in motor vehicles
- Example 2 – variability in the mortality risk to people on the ground from crashing airplanes

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Cost-effectiveness analysis

- One of many tools
- Growing role in medical decision making
- Panel on Cost-effectiveness in Health and Medicine
 - Total costs/Total effectiveness (Incremental ratio)
 - Recommended methods (QALYs, 3% discount rate, societal perspective)
- Typical CEA ignores uncertainty, variability, time, preferences and other attributes, troubles with zeros, criteria for “acceptability”

Why care about dynamic nature?

- Optimal strategies change with time
- Dynamics may be very important to model to characterize the benefits of herd immunity
- Times of major shifts (e.g., perceptions of risk and benefits change going from wild type cases to vaccine-associated cases, with eradication risk shifts to polio in bio warfare)
- When we assess the CE ratio may matter in terms of policy

Changing CE model components

- Most vaccine CEA's assume constant probabilities of getting infection (for both vaccinated and unvaccinated children) – may not capture big herd immunity effects (e.g. mass vaccination reduces risks for unvaccinated as well as vaccinated people)
- Other time-dependent factors:
 - Costs (For single vaccine and program, do these go up, down, or stay the same over time?)
 - Preferences and values
 - Societal dynamics (urbanization, more women working so staying home has greater opportunity costs)
 - Technology

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Do these matter?

- Consider a case study on polio
 - Long history
 - ... but not too long
 - Numerous interventions
 - Near eradication
 - Good time to remind people
 - Story of many successes
 - Could make the transition from ID to possible BW agent if public health community successful

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Project: Background

- Herd immunity effects following polio vaccination.
- E.g. mass vaccination of 95% of infants will reduce the probability for unvaccinated persons as well.
- Other time-dependent factors:
 - price of vaccine
 - with discounting of health and dollars: ->point of time of disease is important
 - demography, technology, etc.

Retrospective Polio CEA Model(1)

- Ideally, we have for all vaccine programs:
 - $\text{Cost}(t) = (V(t)tg(t)vc(t) + (D(t) - D^0(t))H(t))e^{rt}$
 - $\text{Effectiveness}(t) = (D^0(t) - D(t))Q(t)$
- $vc(t)$ = vaccine coverage (as function of time)
 $V(t)$ = vaccine costs per completed vaccine schedule
 $tg(t)$ = target group r = interest to year 2000 dollars
 $D(t)$ = disease burden (incidence) under mass vaccination
 $D^0(t)$ = incidence in absence of immunization program
 $H(t), Q(t)$ = health costs resp. QALYs lost per disease case

Retrospective Polio CEA Model(2)

- Cumulative cost-effectiveness ratio:

$$\text{CCE}(t) = \frac{\text{integrated discounted costs until } t}{\text{integrated discounted health gains until } t}$$

- Cost-effectiveness ratio:

$$\text{CE} = \text{CCE}(T_{\text{end}})$$

Suggested T_{end} : 2015

Retrospective Polio CEA Model(3)

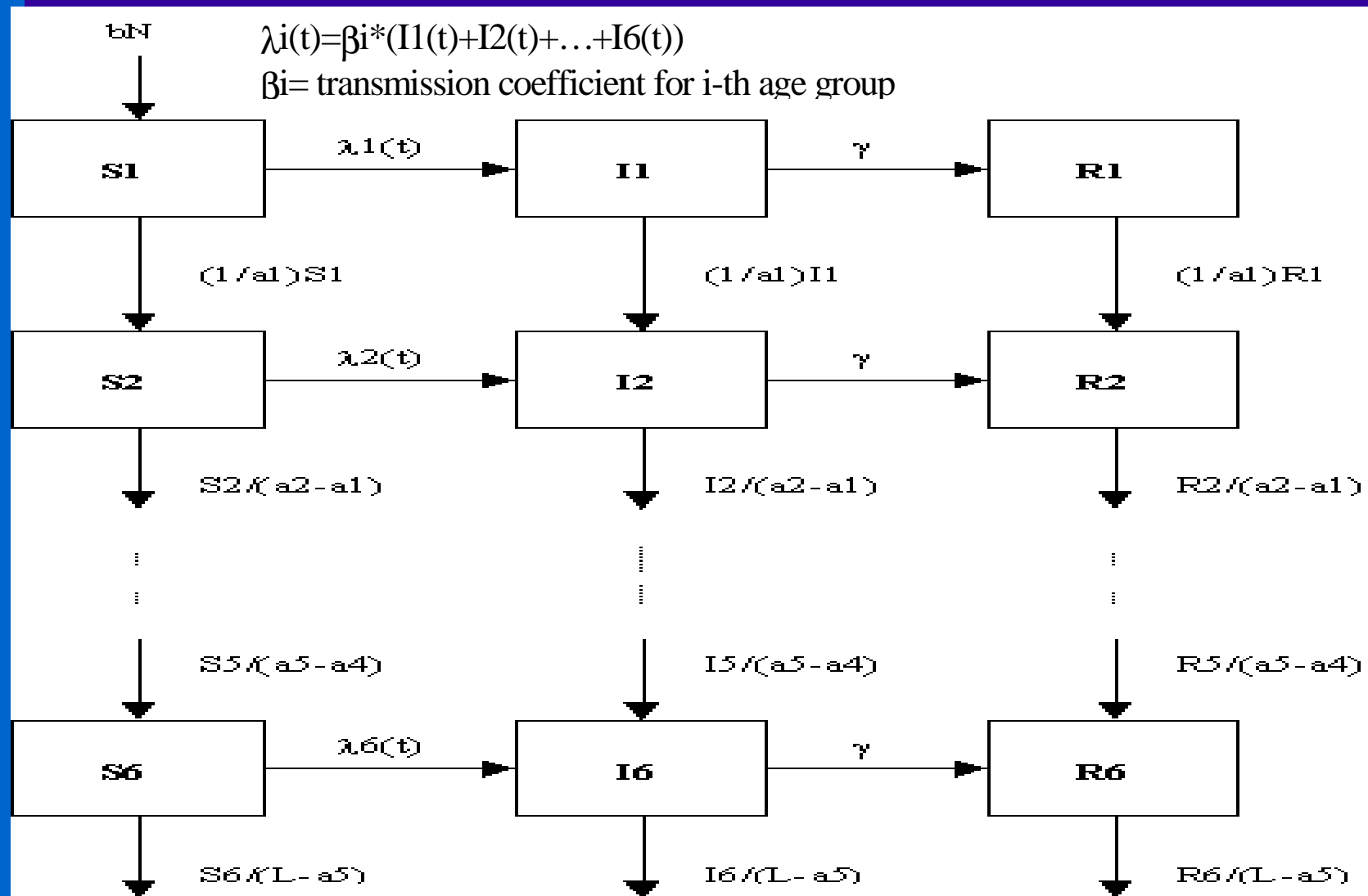
- The disease incidence with or without immunization program can be calculated with a transmission model -> requiring assumptions about transmission, and data
- For every variable except incidence, real historic data will be used.

Concept of Transmission

Models: SIR Models

- $S(t)$ = number of *susceptibles*: those individuals that could get infection
- $I(t)$ = number of *infecteds*: those that are infectious: they can contaminate susceptibles
- $R(t)$ = number of *removeds*: those that are immune to infection (*recovereds, resistants*)
- Transition rates between S, I, R \rightarrow differential eqns.
- $\lambda(t) = \beta * I(t)$ = force of infection = per susceptible rate of infection, β is the *transmission coefficient*

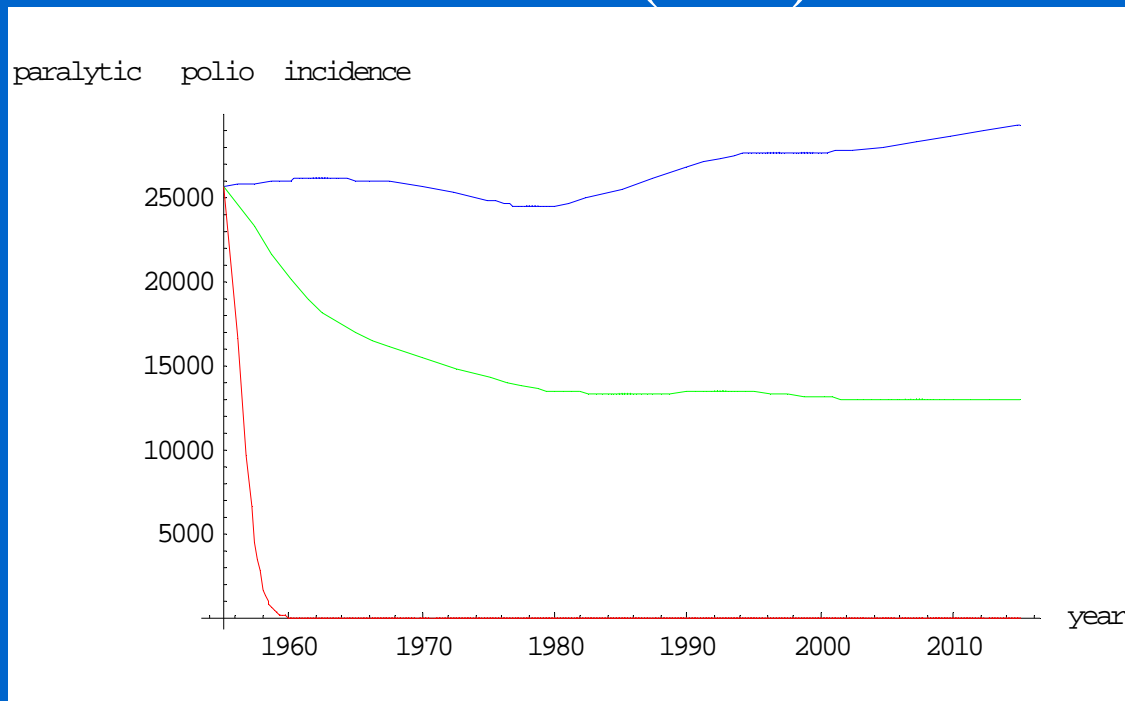
Transmission model (1)



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-
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Example Results

Vaccine 1, paralytic polio incidence with static(green) and dynamic(red) transmission model and without vaccine(blue):



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Insights

- Risk analysis and decision analysis tools have evolved to the point where they are helpful in characterizing and understanding the trade-offs associated with tough choices
- Must consider the dynamics of the disease to accurately quantify the health benefits
- Complex problem – analysis is needed
 - No zero risk
 - Real trade-offs

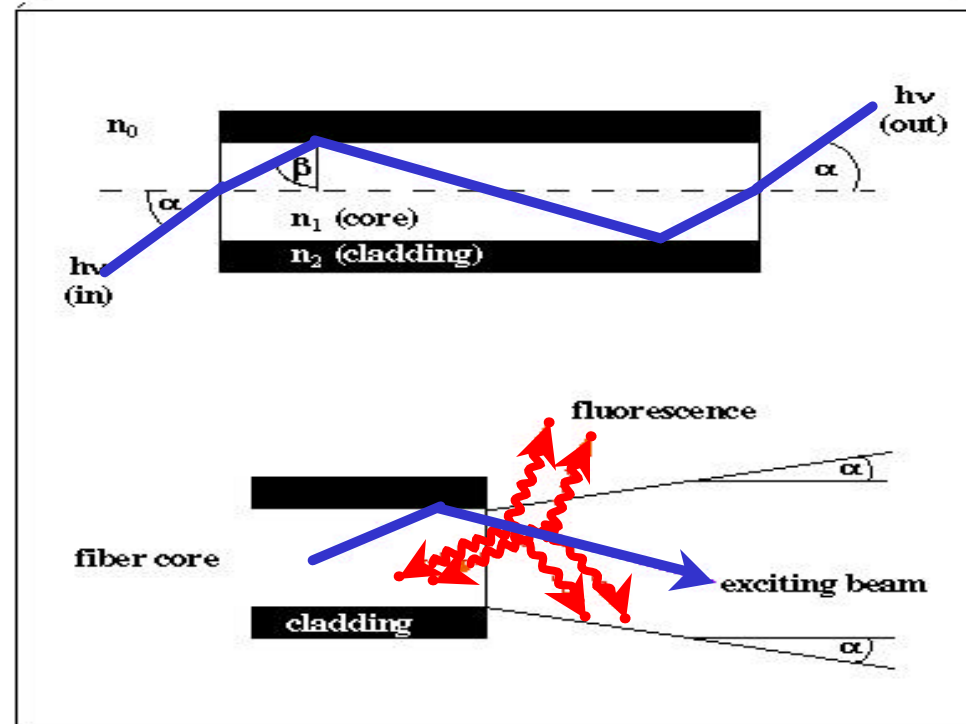
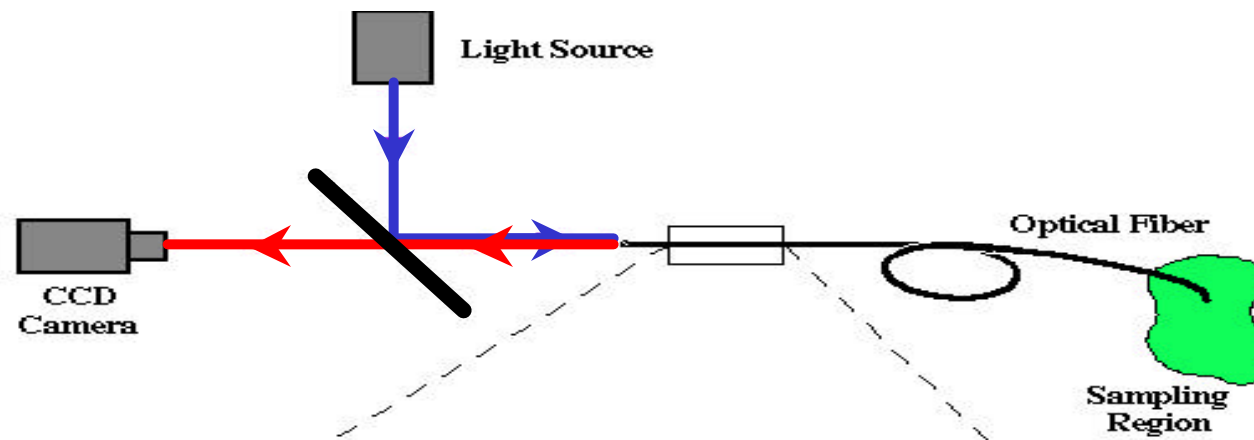
Microarrays

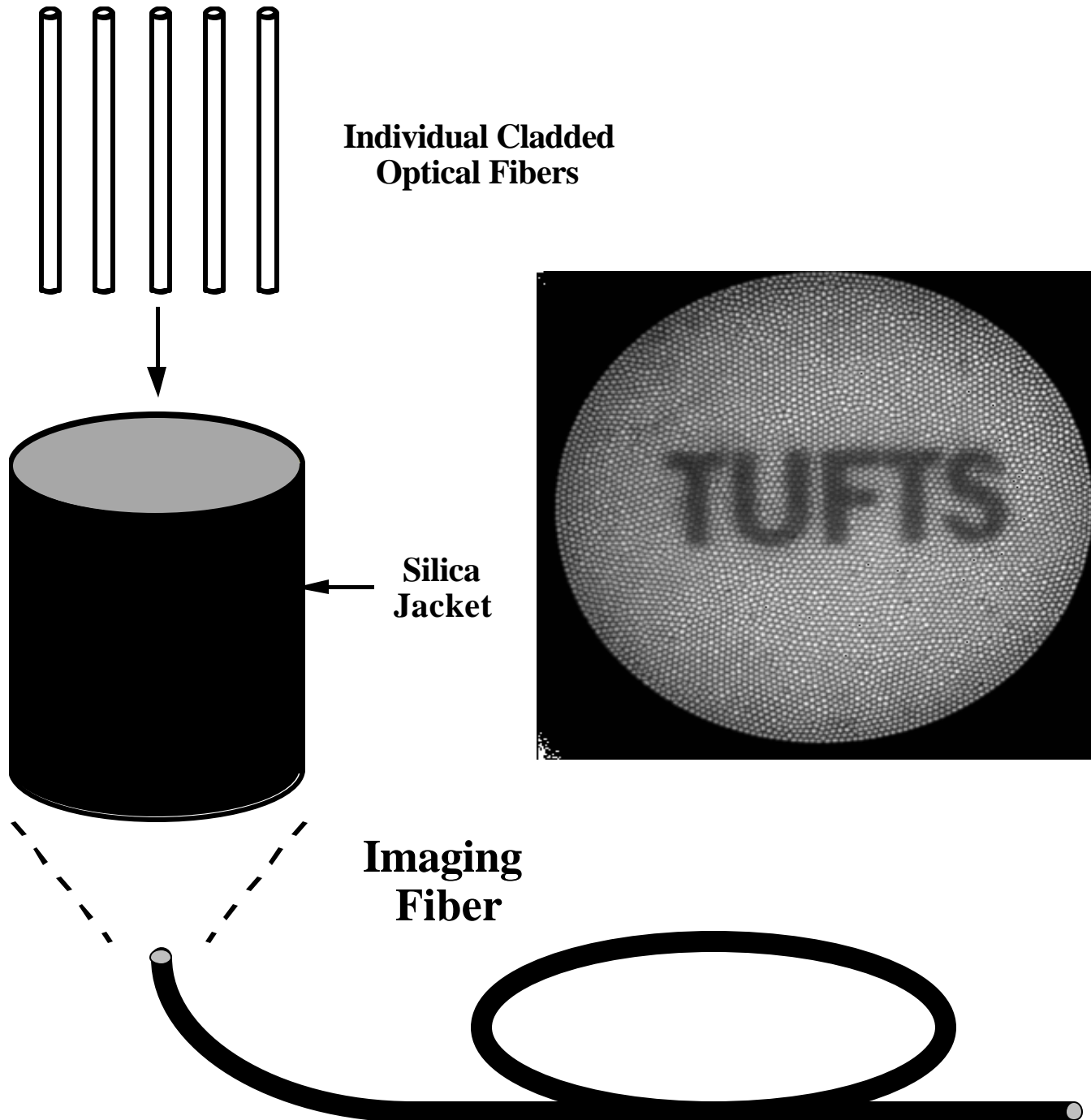
New England Bioterrorism Preparedness Workshop

Dr. David Walt

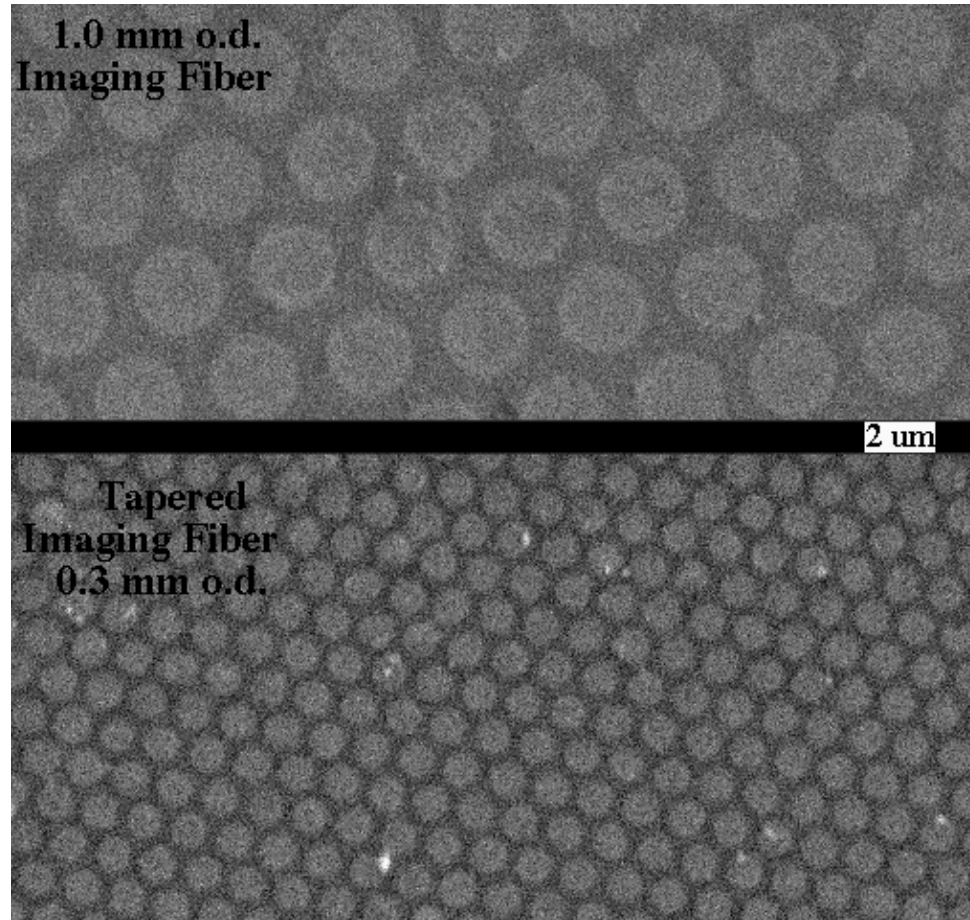
Tufts University

4 April 2002





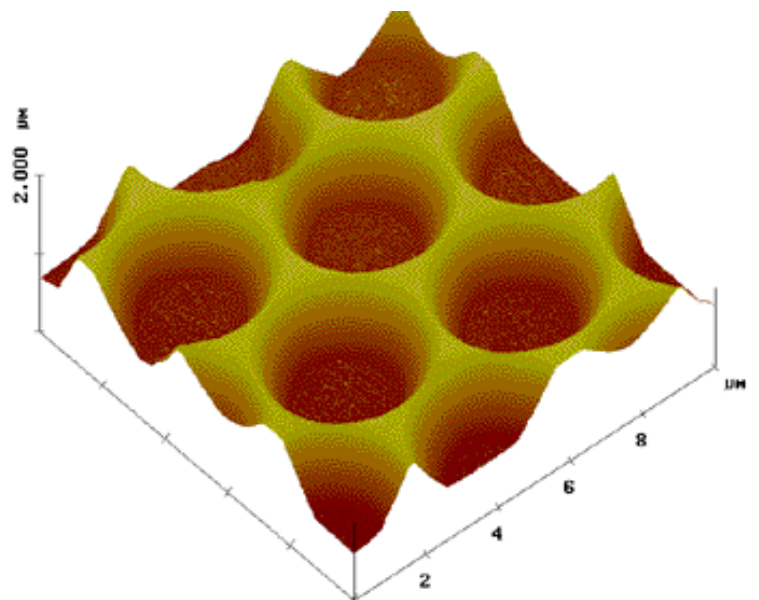
Optical Imaging Fiber Before and After Tapering



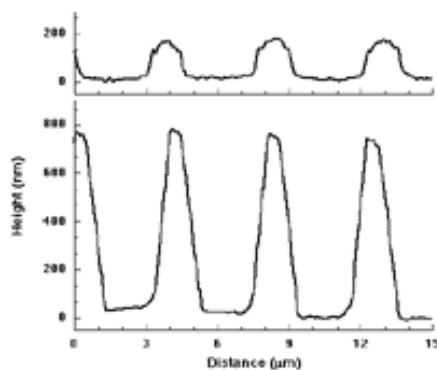
**Individual Core
Diameter ~ 2.6 μ m**

**Individual Core
Diameter ~ 0.85 μ m**

AFM of a Chemically-Etched 1000- μ m Diameter Imaging Fiber



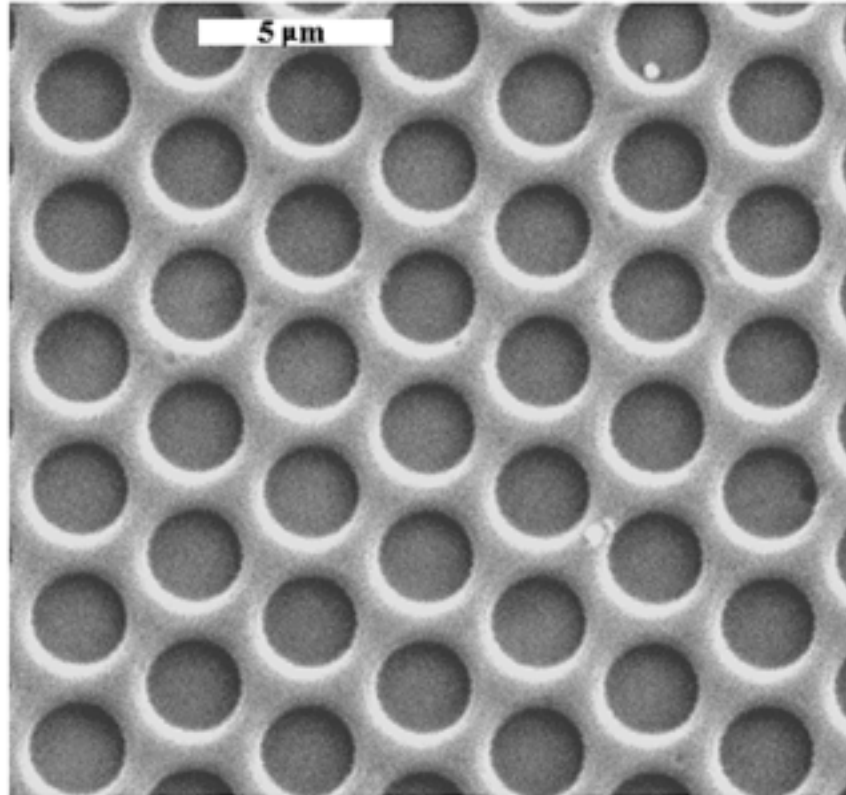
Well Profiles



15 s etch

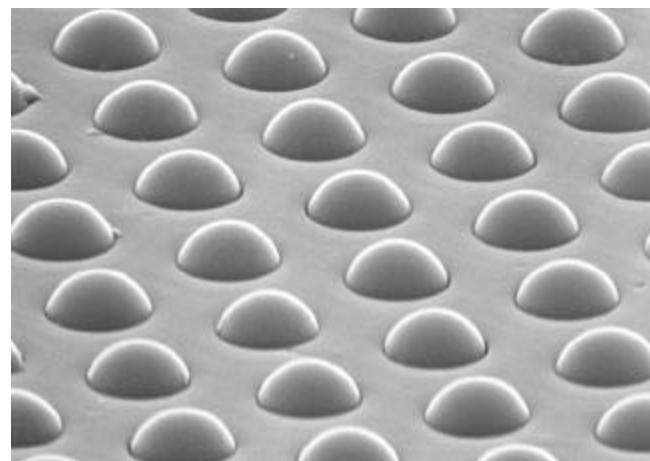
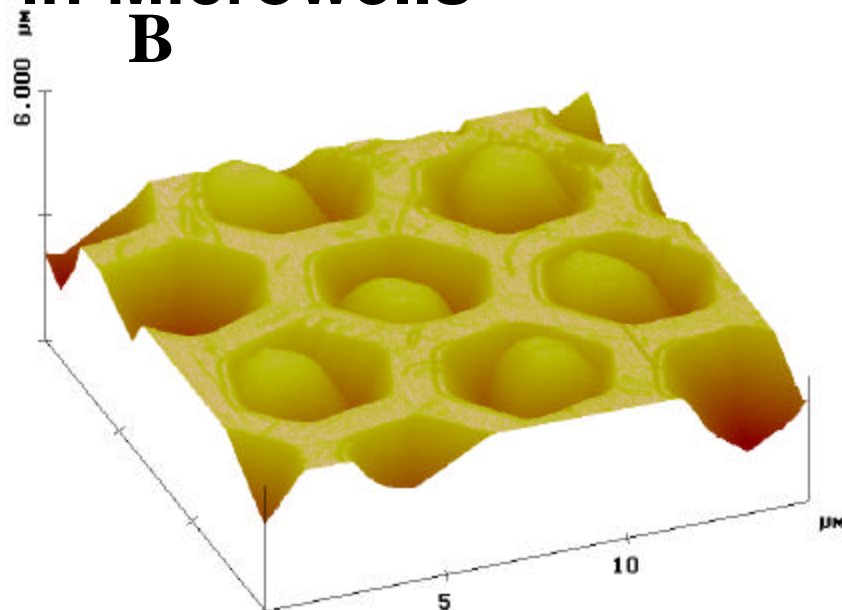
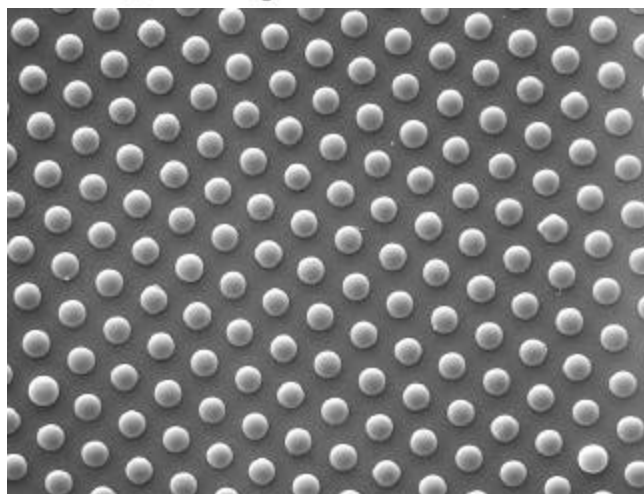
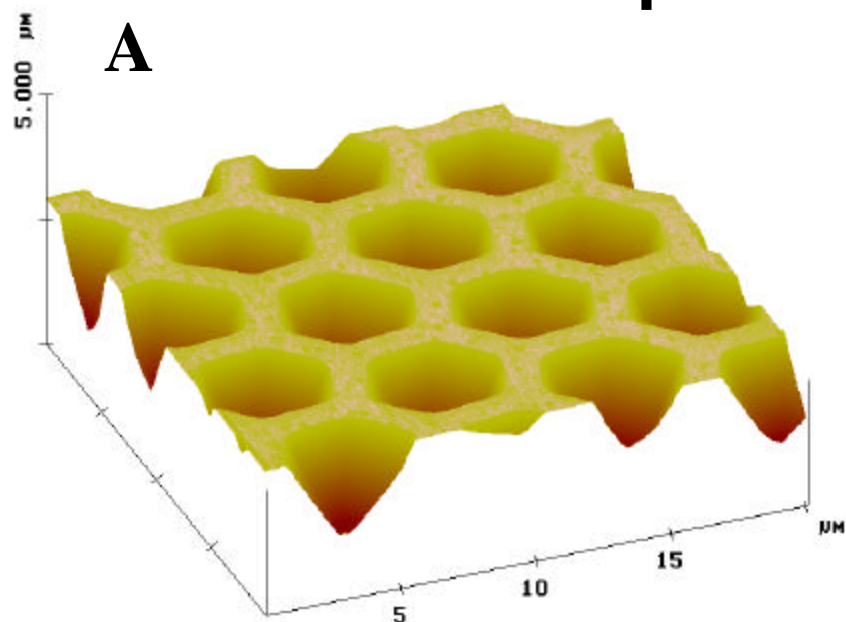
60 s etch

SEM of a Chemically-Etched 1000- μ m Diameter Imaging Fiber

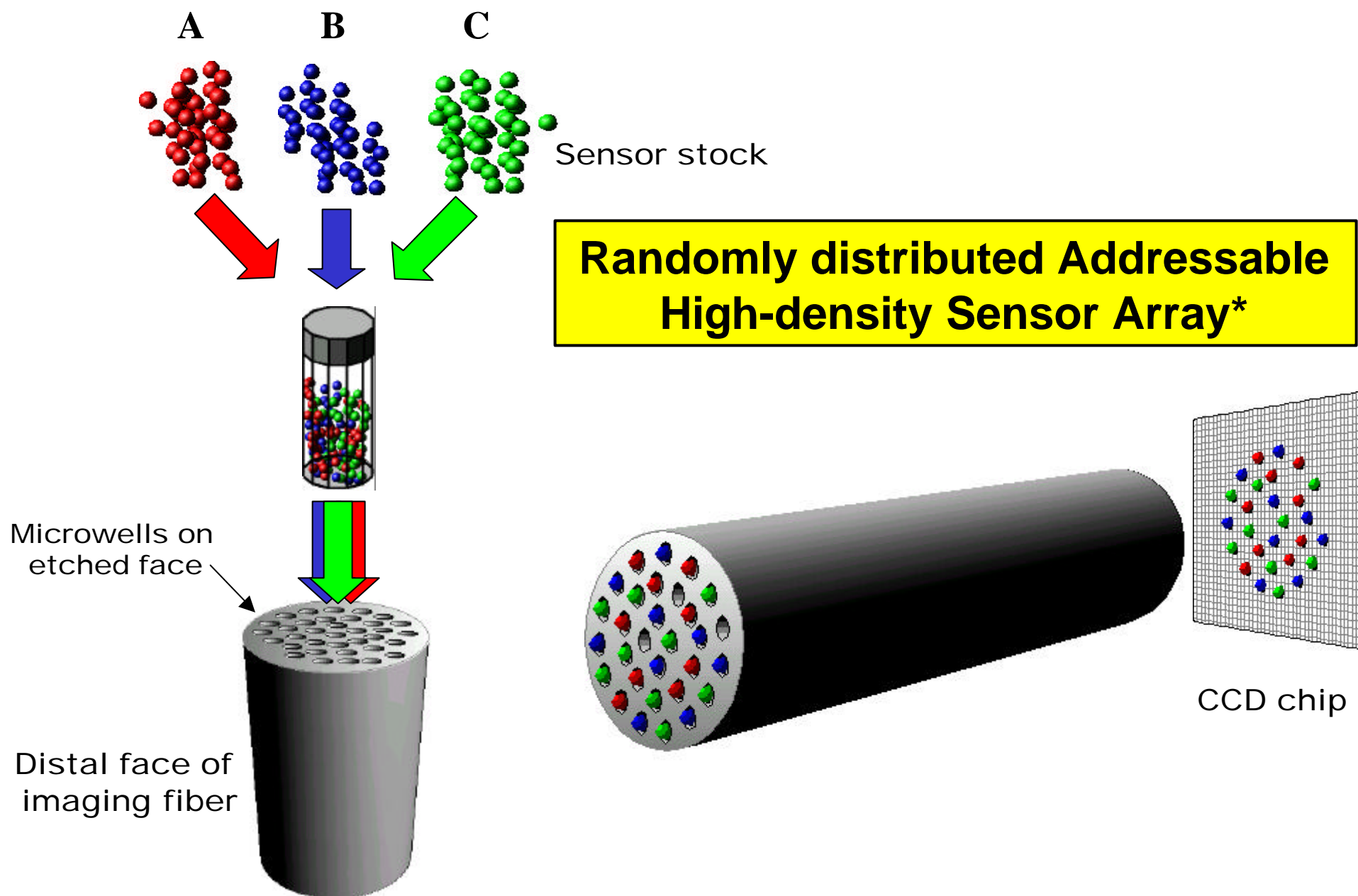


Pantano, P.; Walt, D.R. *Chem. Mater.* **1996**, 8, 2832-2835

Microspheres in Microwells

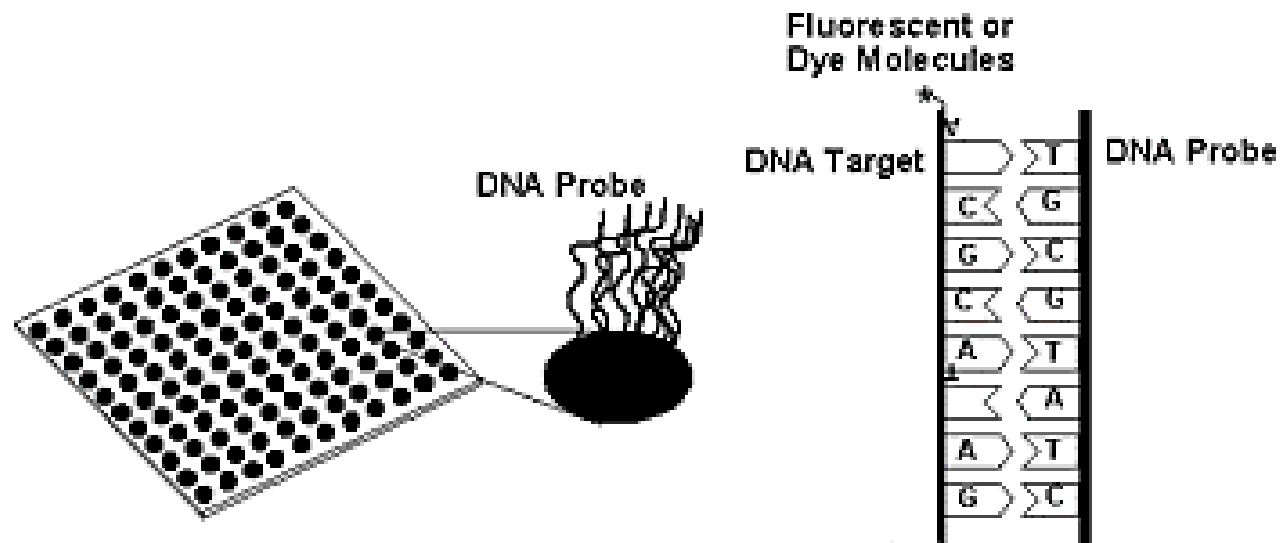


Michael, K.L. *et al. Anal. Chem.* 70 (7): 1242-1248 (1998).

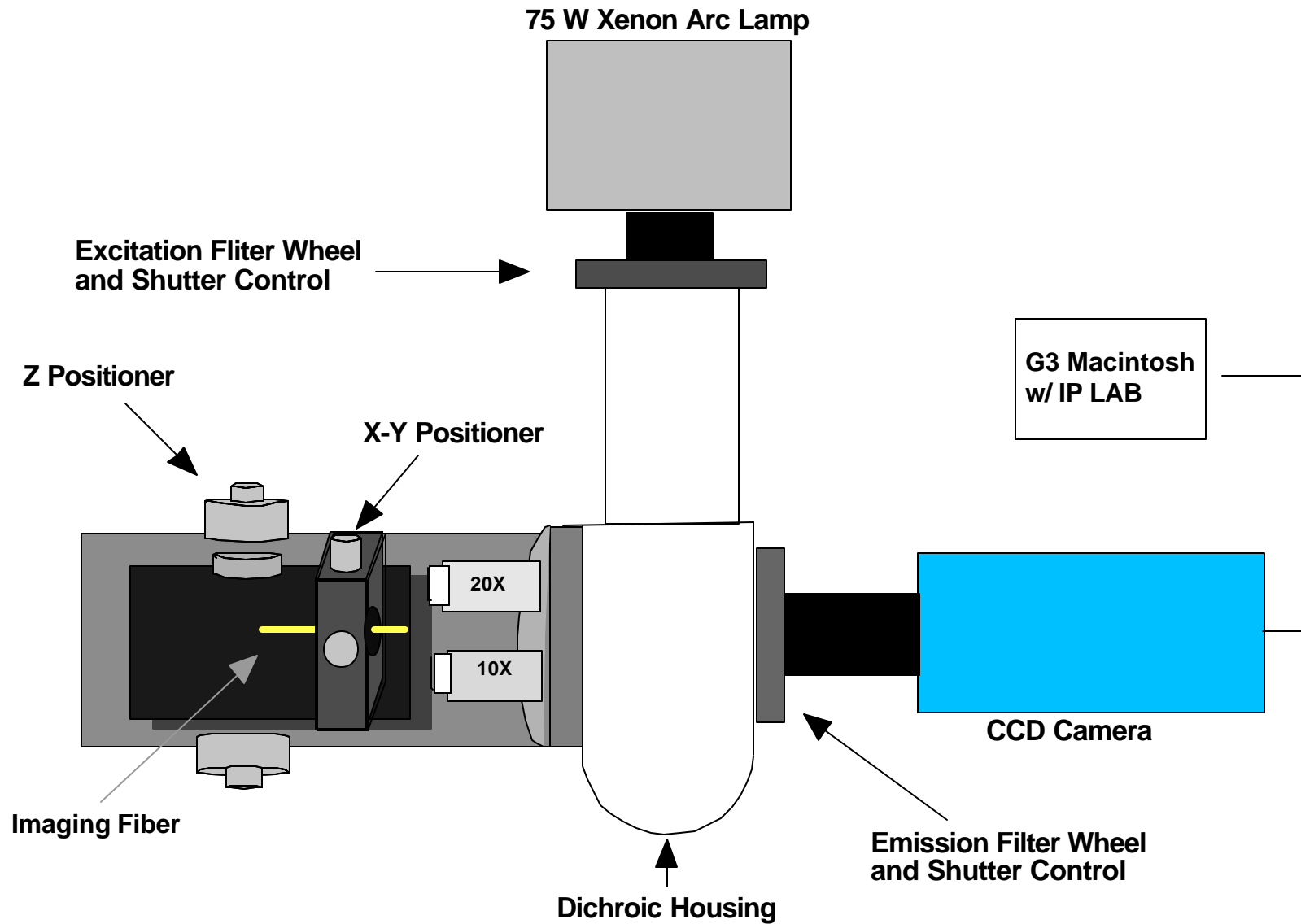


*Michael et al. **1998** *Anal. Chem.* **70**: 1242-1248

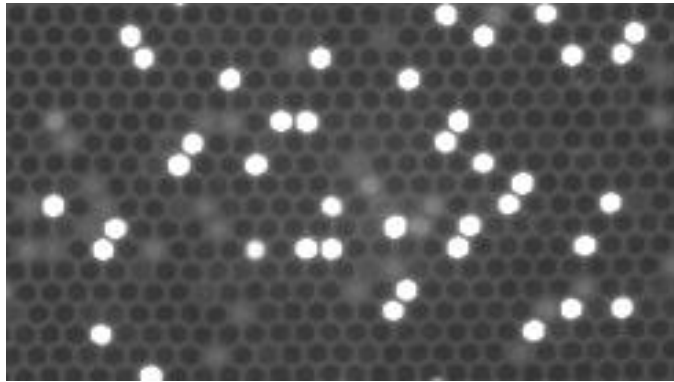
DNA Array Principle



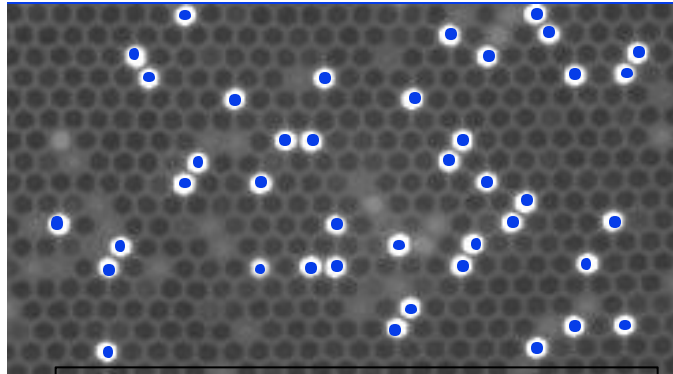
Instrumentation: Modified Fluorescence Microscope



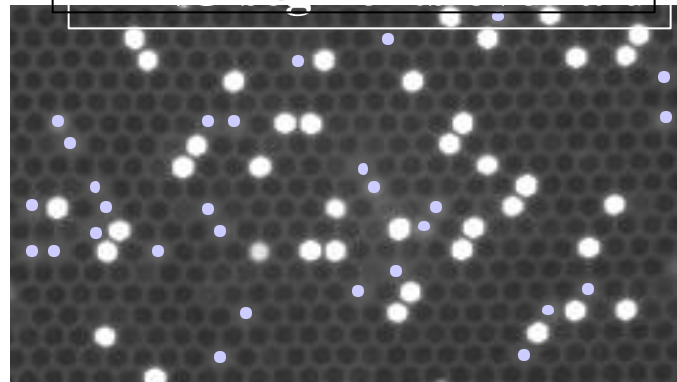
Encoding Signal of Dye 1



Signal 530

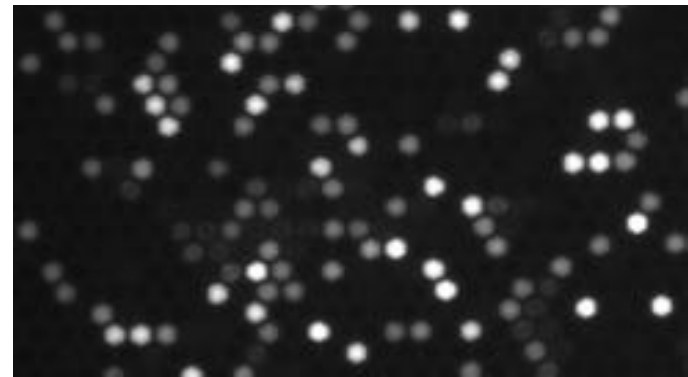


IFNG segments overlaid

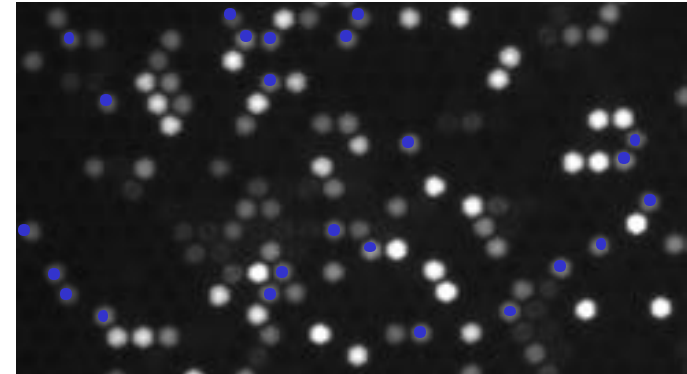


IL2 segments overlaid

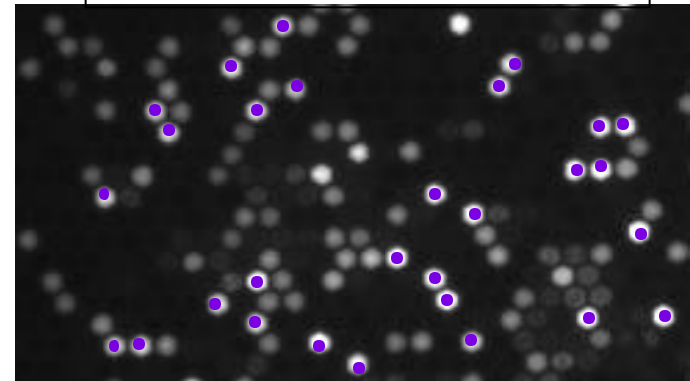
Encoding Signal of Dye 2



Signal 620



Bglo segments overlaid



Hwt segments overlaid

Sequences of 25 Probes used together in a Microsphere Array

1) ?-glo (segment of human ?-globin)²⁶
TCA ACT TCA TCC ACG TTC ACC

2) IFNG (interferon gamma 1)²⁶
IFNG TGG GTT CTC TTG GCT GTT ACT

3) IL2 (interleukin-2)²⁶
TA CAA GAA TCC CAA ACT CAC CAG

4) IL4 (interleukin-4)²⁶
CC AAC TGC TTC CCC CTC TGT

5) IL6 (interleukin-6)²⁶
GT TGG GTC AGG GGT GGT TAT T

6) K-ras WT²⁷
GGA GCT GGT GGC GTA

7) H-ras WT²⁷
CCG GCG GTG T

8) CFTR (cystic fibrosis exon 11)¹³
CAT TAT ACT TGT AGA G

9) R553X (cystic fibrosis exon 10)¹³
TGT AGA ATT ATC TTC

10) PAN132¹⁶ (human peripheral lymphocyte)
CCT CTA TAC TTT AAC GTC AAG

11) Schena-2¹⁶
AAG TTT AAC CTA TAC CCT GTC

12) Hakala-1²⁰
CCT ATG ATG AAT ATA G

13) Hakala-2²⁰
AAT ATG ATA ATG GCC T

14) complement to probe 1
TG AAC GTG GAT GAA GTT G

15) complement to probe 2
AG TAA CAG CCA AGA GAA CCC AAA

16) complement to probe 3
CT GGT GAG TTT GGG ATT CTT GTA

17) complement to probe 4
AC AGA GGG GGA AGC AGT TGG

18) complement to probe 5
AA TAA CCA CCC CTG ACC CAA C

19) complement to probe 6
TAC GCC ACC AGC TCC

20) complement to probe 7
ACA CCG CCG G

21) complement to probe 8
CTC TAC AAG TAT AAT G

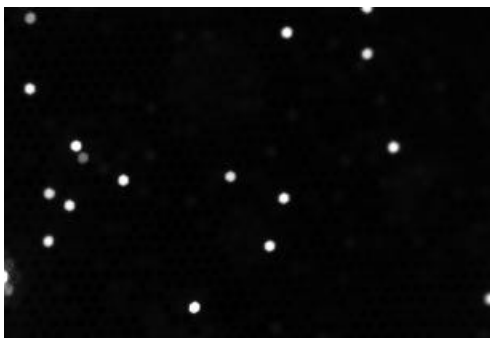
22) complement to probe 9
GAA GAT GTT AAA GTA TAG AGG

23) complement to probe 10
CTA GAC GTT AAA GTA TAG AGG

24) complement to probe 12
CTA TAT TCA TCA TAG G

25) complement to probe 13
AGG CCA TTA TCA TAT T

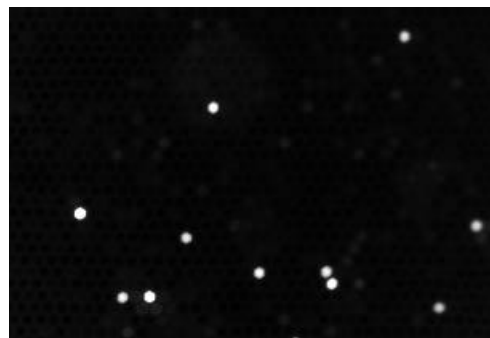
2



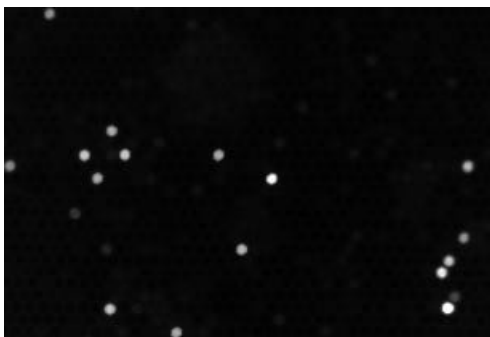
15



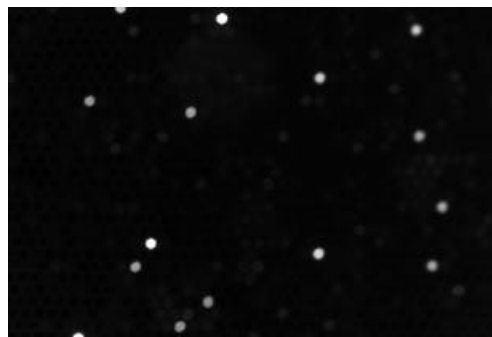
14



3



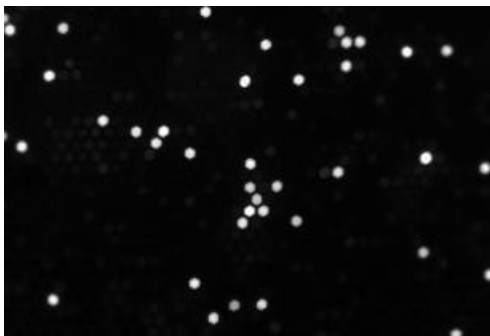
18



11



6



23



9



8



24




5

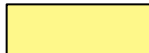


E. coli Allelic Discrimination

ycgW locus*

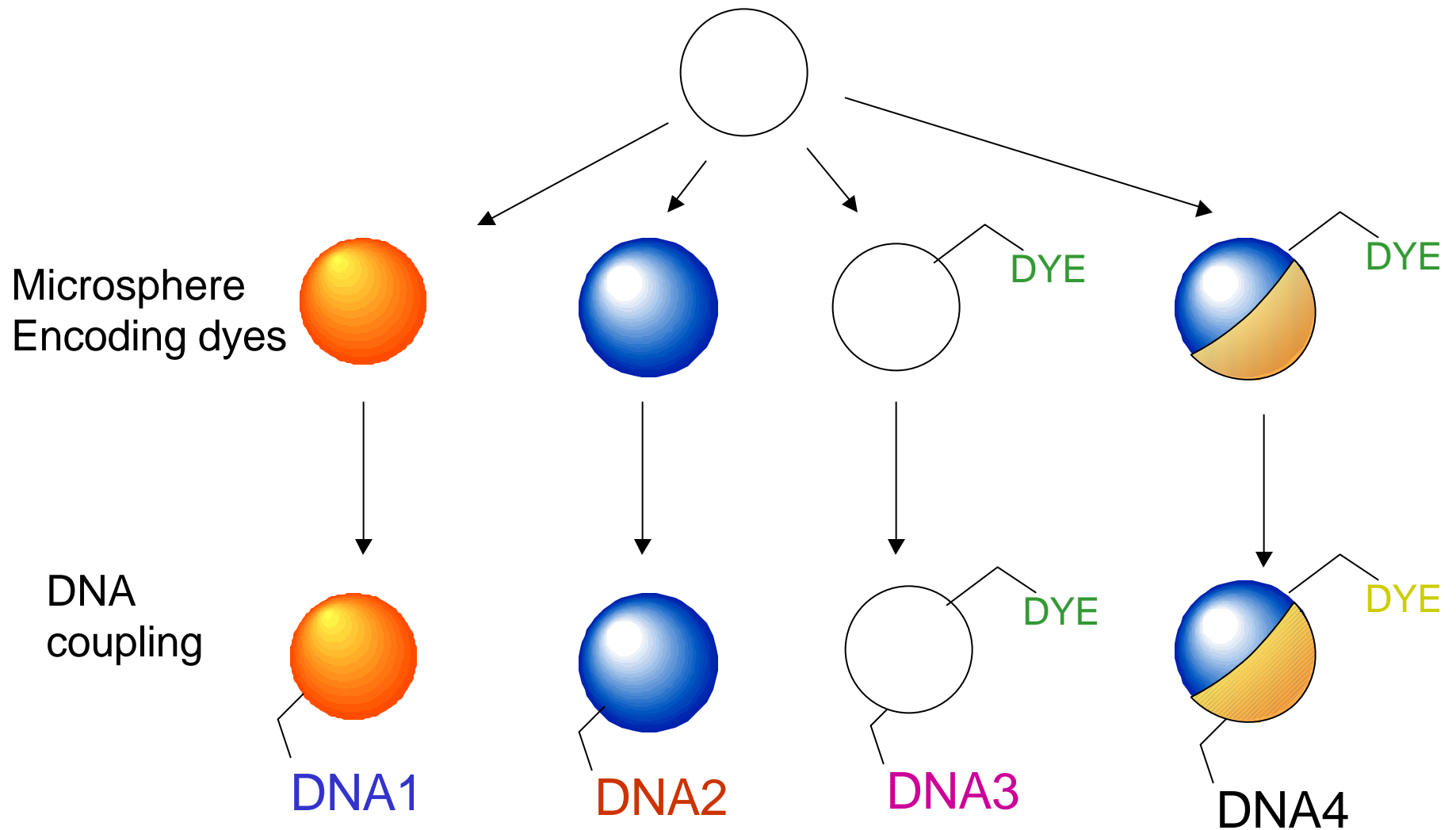
| No. | strain | strain sequence | | | | | | | | | | No. | strain | strain sequence | | | | | | | | |
|-----|----------|---------------------------|--|--|--|--|--|--|--|--|--|-----|----------|---------------------------|--|--|--|--|--|--|--|--|
| 1 | BSR9b | T T T T T G A A G G G G G | | | | | | | | | | 19 | HER1265 | no PCR product | | | | | | | | |
| 2 | BSR9c | T T T T T G A A G G G G G | | | | | | | | | | 20 | HER1266 | no PCR product | | | | | | | | |
| 3 | "ETEC" | T T T T T T G A A G G G G | | | | | | | | | | 21 | EC68 | no PCR product | | | | | | | | |
| 4 | O111NM | T T T T T G A A G G G G G | | | | | | | | | | 22 | EC69 | T T T T T T G A A G G G G | | | | | | | | |
| 5 | O113:H2 | T T T T T G A A G G G G G | | | | | | | | | | 23 | EC63 | no PCR product | | | | | | | | |
| 6 | O157NM | T T T T T T G A A G G G G | | | | | | | | | | 24 | EC54 | no PCR product | | | | | | | | |
| 7 | HER1058 | no PCR product | | | | | | | | | | 27 | O86:H10 | T T T T T G A A G G G G G | | | | | | | | |
| 8 | K12DH5a | T T T T T T G A A G G G G | | | | | | | | | | 37 | O86:H18 | T T T G T T T T T T T G | | | | | | | | |
| 9 | K12W4100 | T T T T T T G A A G G G G | | | | | | | | | | 30 | O8:H9 | T T T T T G A A G G G G G | | | | | | | | |
| 10 | O55:H7 | T T T T C G A A G G G G G | | | | | | | | | | 34 | O9:H33 | T T T T T T G A A G G A G | | | | | | | | |
| 11 | "EPEC" | T T T T T T G A A G G G G | | | | | | | | | | 38 | O153:H- | T T T T T T G A A G G G G | | | | | | | | |
| 12 | K12W3110 | T T T T T T G A A G G G G | | | | | | | | | | 43 | O26:H11 | T T T T T G A A G G G G G | | | | | | | | |
| 13 | O22:H8 | T T T T T G A A G G G G G | | | | | | | | | | 48 | O127:H21 | T T T T T G A A G G G G G | | | | | | | | |
| 14 | O26:H- | T T T T T T G A A G G G G | | | | | | | | | | 52 | EC1 | T T T T T T G A A G G G G | | | | | | | | |
| 15 | O42:H2 | T T T T T G A A G G G G G | | | | | | | | | | 53 | EC7 | T T T T T T G A A G G G G | | | | | | | | |
| 16 | O157:H7 | no PCR product | | | | | | | | | | 54 | EC18 | T T T T T T G A A G G A G | | | | | | | | |
| 17 | HER1057 | no PCR product | | | | | | | | | | 55 | EC47 | T T T G T T T T T T T G G | | | | | | | | |
| 18 | HER1261 | no PCR product | | | | | | | | | | 56 | EC52 | no PCR product | | | | | | | | |
| | Cons. | T T T * * * * * * * * * G | | | | | | | | | | | Cons. | T T T * * * * * * * * * G | | | | | | | | |

 = probe 1 signal

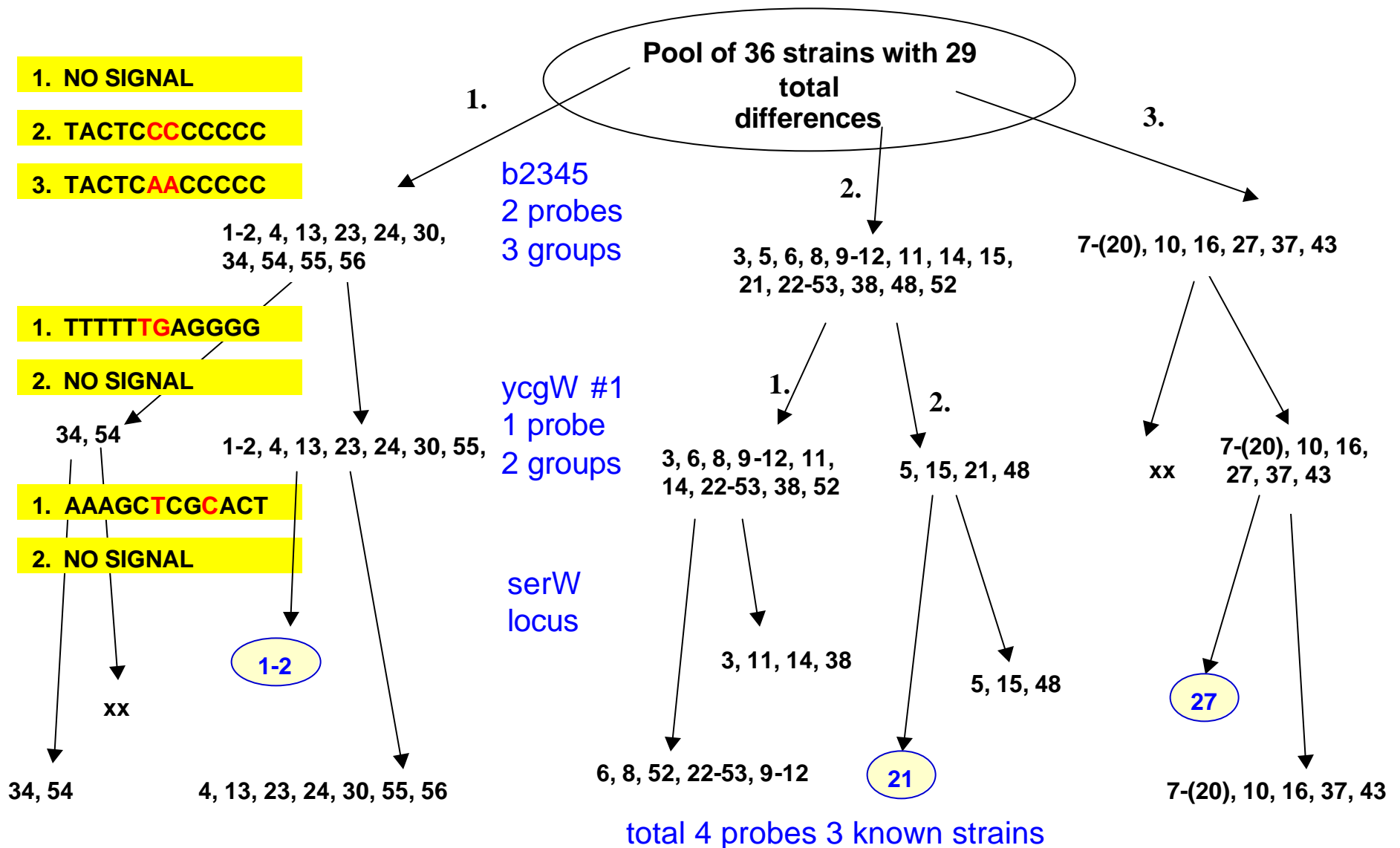
 = no signal

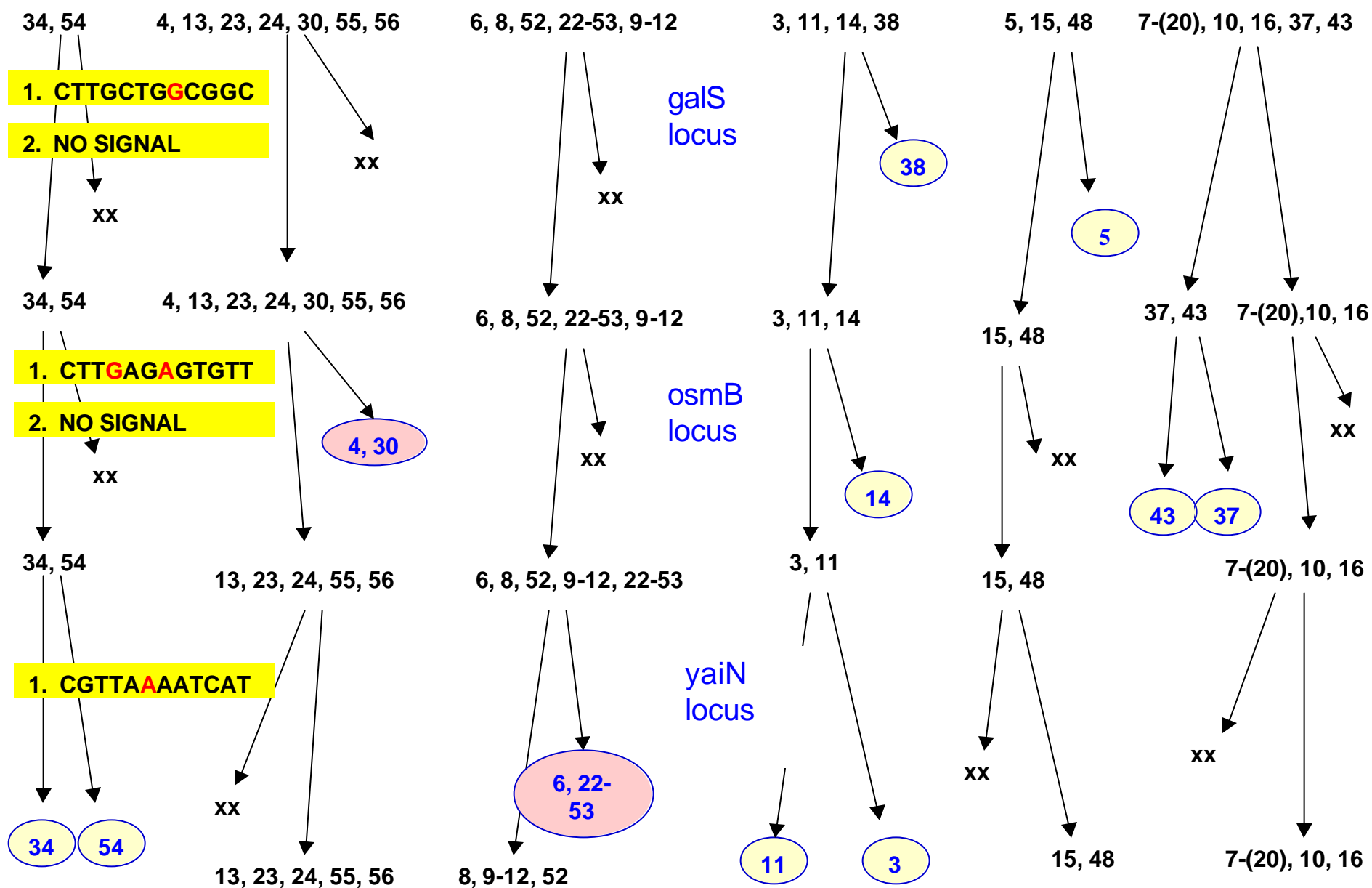
*ycgW locus is 77 nucleotides long

Microsphere Functionalization

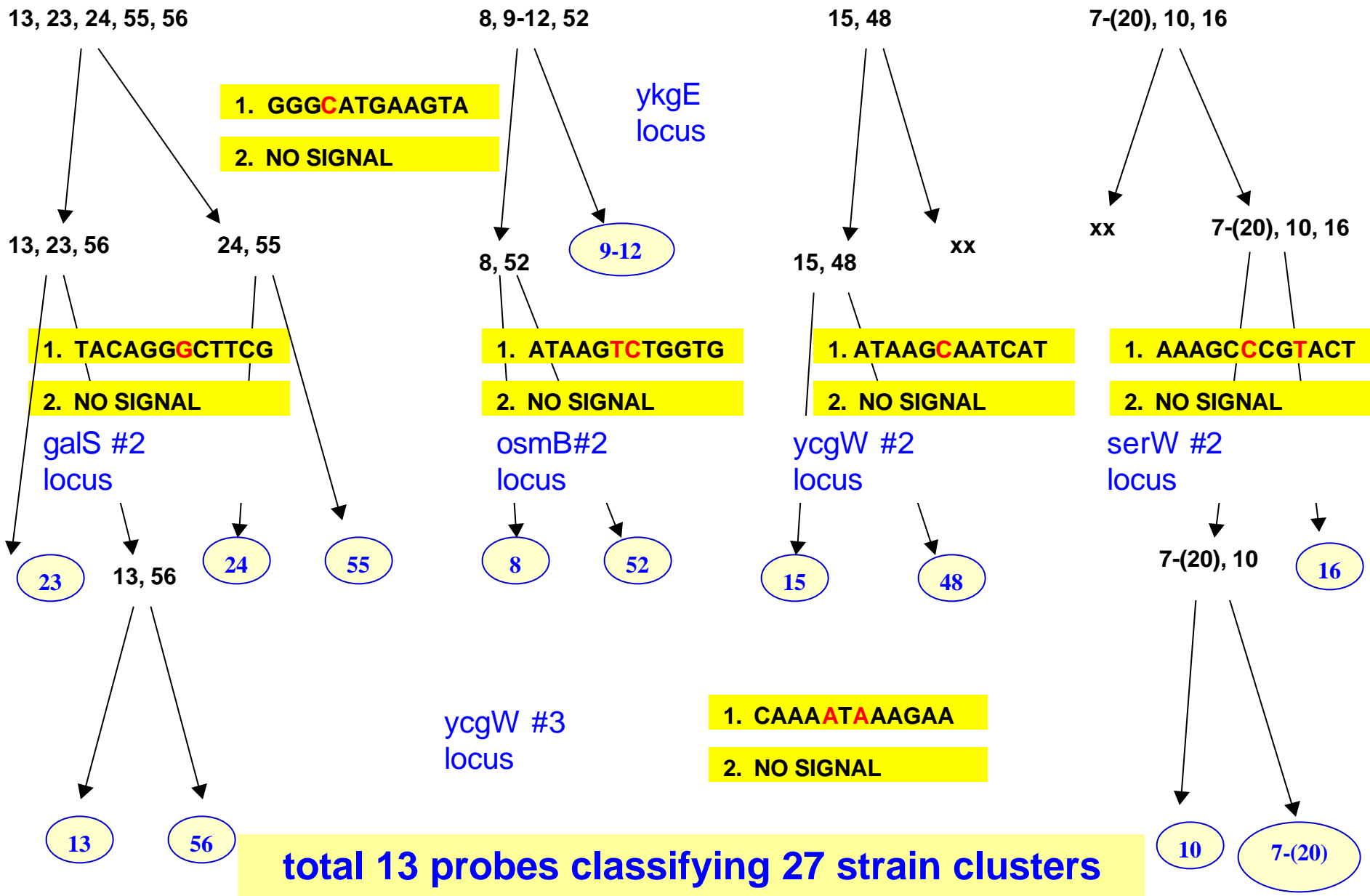


E. coli Genomic Discrimination Flowchart





total 7 probes 12+ known strains

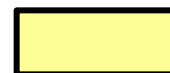


E. coli Genomic Pattern Response

| No. | strain | ycgW | serW | osmB | yaiN | ykgE |
|-----|----------|------|------|------|------|------|
| 14 | O26:H- | | | | | |
| 11 | "EPEC" | | | | | |
| 3 | "ETEC" | | | | | |
| 6 | O157NM | | | | | |
| 22 | EC69 | | | | | |
| 9 | K12W4100 | | | | | |
| 12 | K12W3110 | | | | | |
| 8 | K12DH5a | | | | | |



= signal response



= no signal

E. coli Genomic Pattern Response

| No. | strain | ycgW | b2345 | serW | galS | Osmb | yKgE | ycgW#3 | serW#2 |
|-----|---------|------|-------|------|------|------|------|--------|--------|
| 21 | EC68 | | | | | | | | |
| 1 | BSR9b | | | | | | | | |
| 2 | BSR9c | | | | | | | | |
| 15 | O42:H2 | | | | | | | | |
| 5 | O113:H2 | | | | | | | | |
| 4 | O111NM | | | | | | | | |
| 24 | EC54 | | | | | | | | |
| 13 | O22:H8 | | | | | | | | |
| 23 | EC63 | | | | | | | | |
| 10 | O55:H7 | | | | | | | | |
| 16 | O157:H7 | | | | | | | | |
| 7 | HER1058 | | | | | | | | |
| 17 | HER1057 | | | | | | | | |
| 18 | HER1261 | | | | | | | | |
| 19 | HER1265 | | | | | | | | |
| 20 | HER1266 | | | | | | | | |

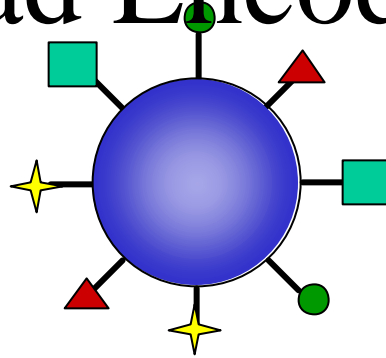


= signal response

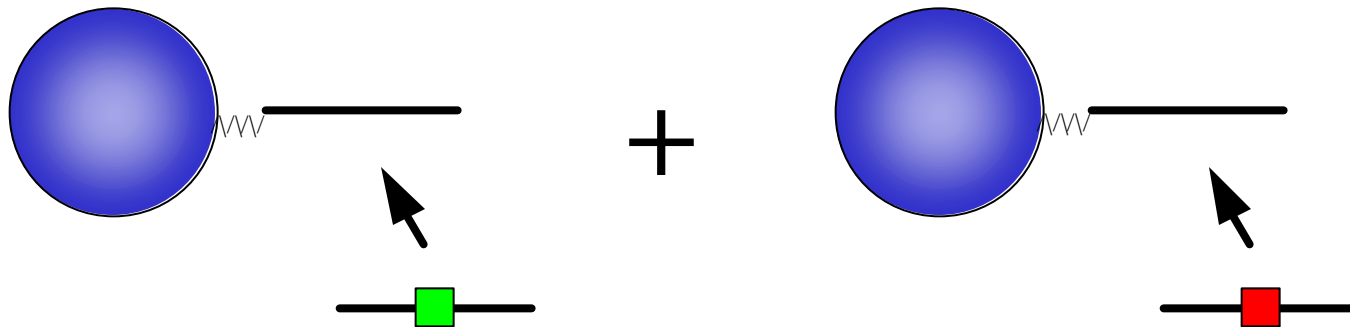


= no signal

Bead Encoding



Sequential Decoding



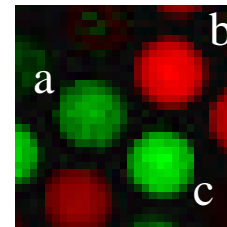
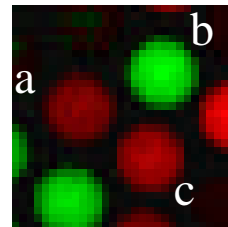
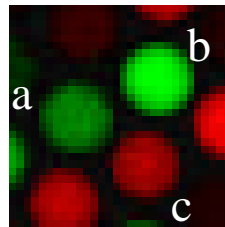
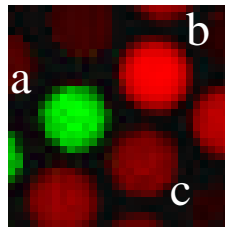
Decoding 16 Probes

Hyb # 1

2

3

4



| | Hyb.#1 | Hyb.#2 | Hyb.#3 | Hyb.#4 |
|---|--------|--------|--------|--------|
| a | green | green | red | green |
| b | red | green | green | red |
| c | red | red | red | green |

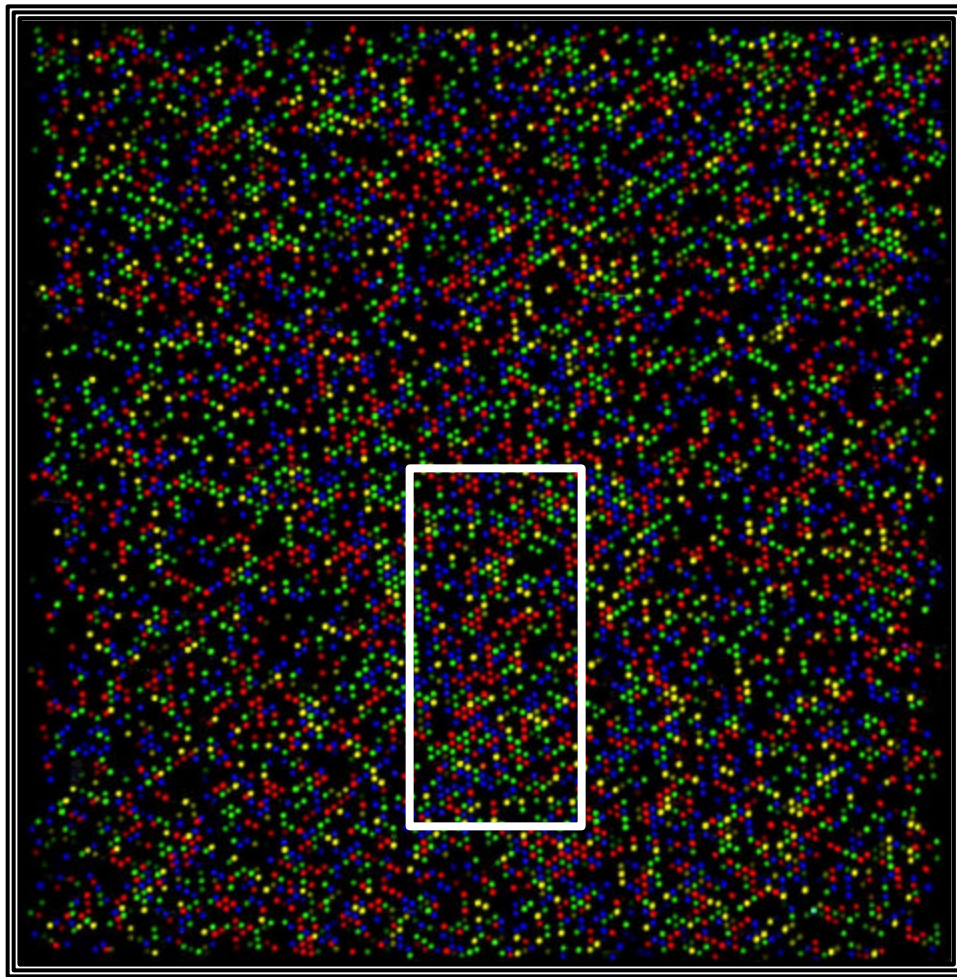
| | 1 | 2 | 3 | 4 | Sequence |
|---|-------|-------|-------|-------|----------|
| ← | green | green | green | green | 1 |
| ← | green | green | green | red | 2 |
| ← | green | green | red | green | 3 |
| ← | green | green | red | red | 4 |
| ← | green | red | green | green | 5 |
| ← | green | red | green | red | 6 |
| ← | green | red | red | green | 7 |
| ← | green | red | red | red | 8 |
| ← | red | green | green | green | 9 |
| ← | red | green | green | red | 10 |
| ← | red | green | red | green | 11 |
| ← | red | green | red | red | 12 |
| ← | red | red | green | green | 13 |
| ← | red | red | green | red | 14 |
| ← | red | red | red | green | 15 |
| ← | red | red | red | red | 16 |

Decoding is Exponential

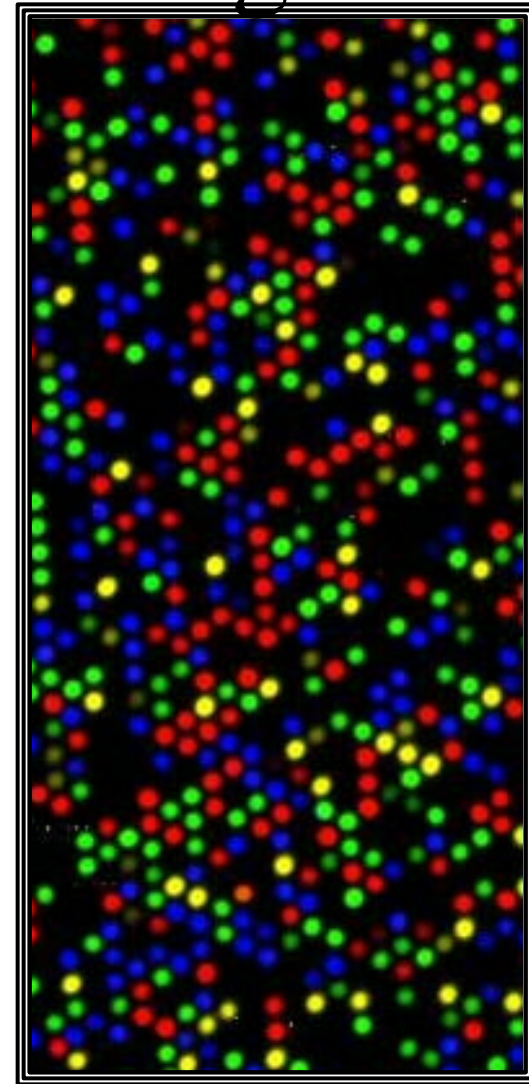
x labels , z steps = x^z codes

- 2 Dyes ^ 4 Steps = 16 Codes
- 4 Dyes ^ 6 Steps = 4,096 Codes

Four-Color Decoding

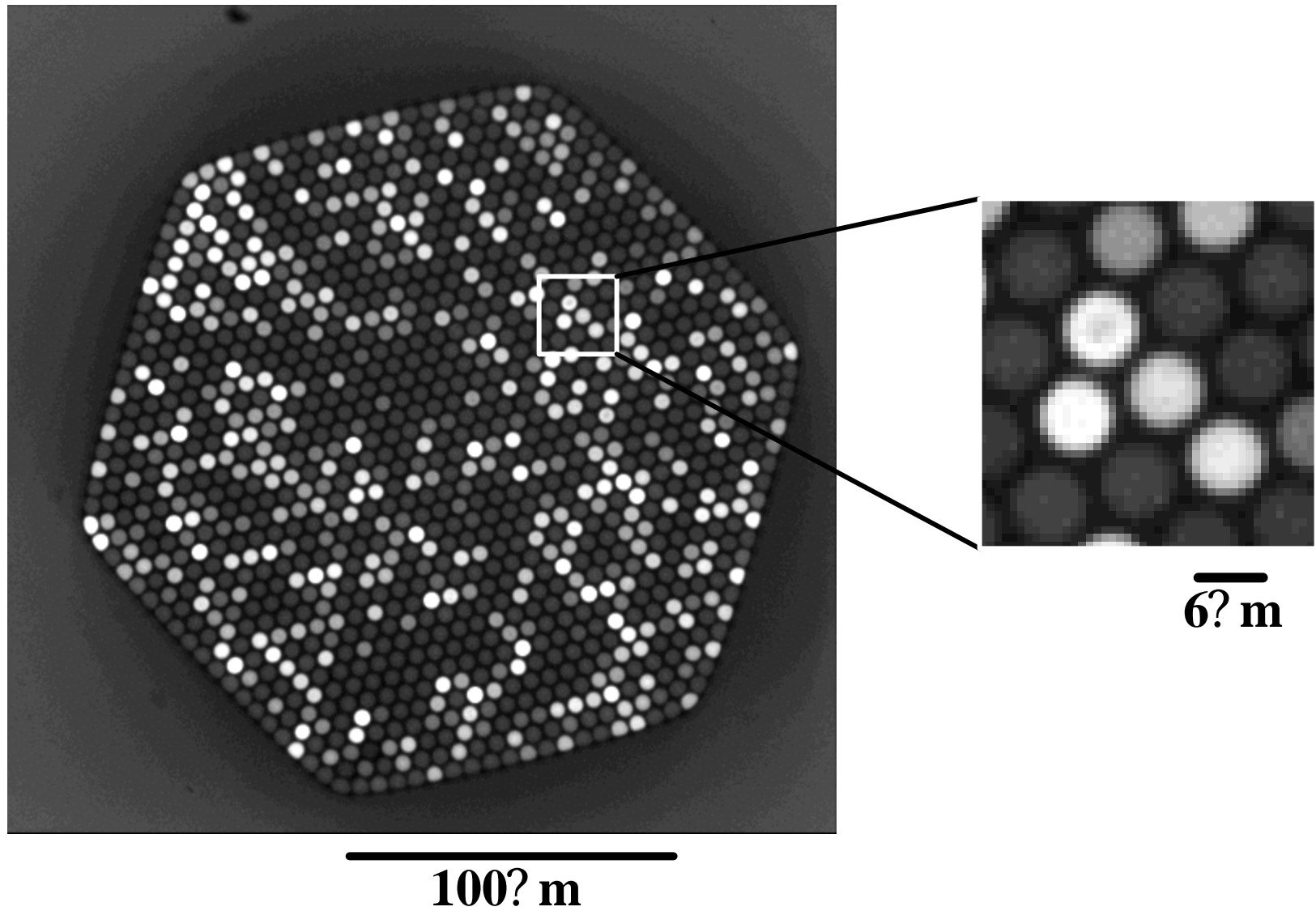


~13,000 Wells, 16 Probe Sequences

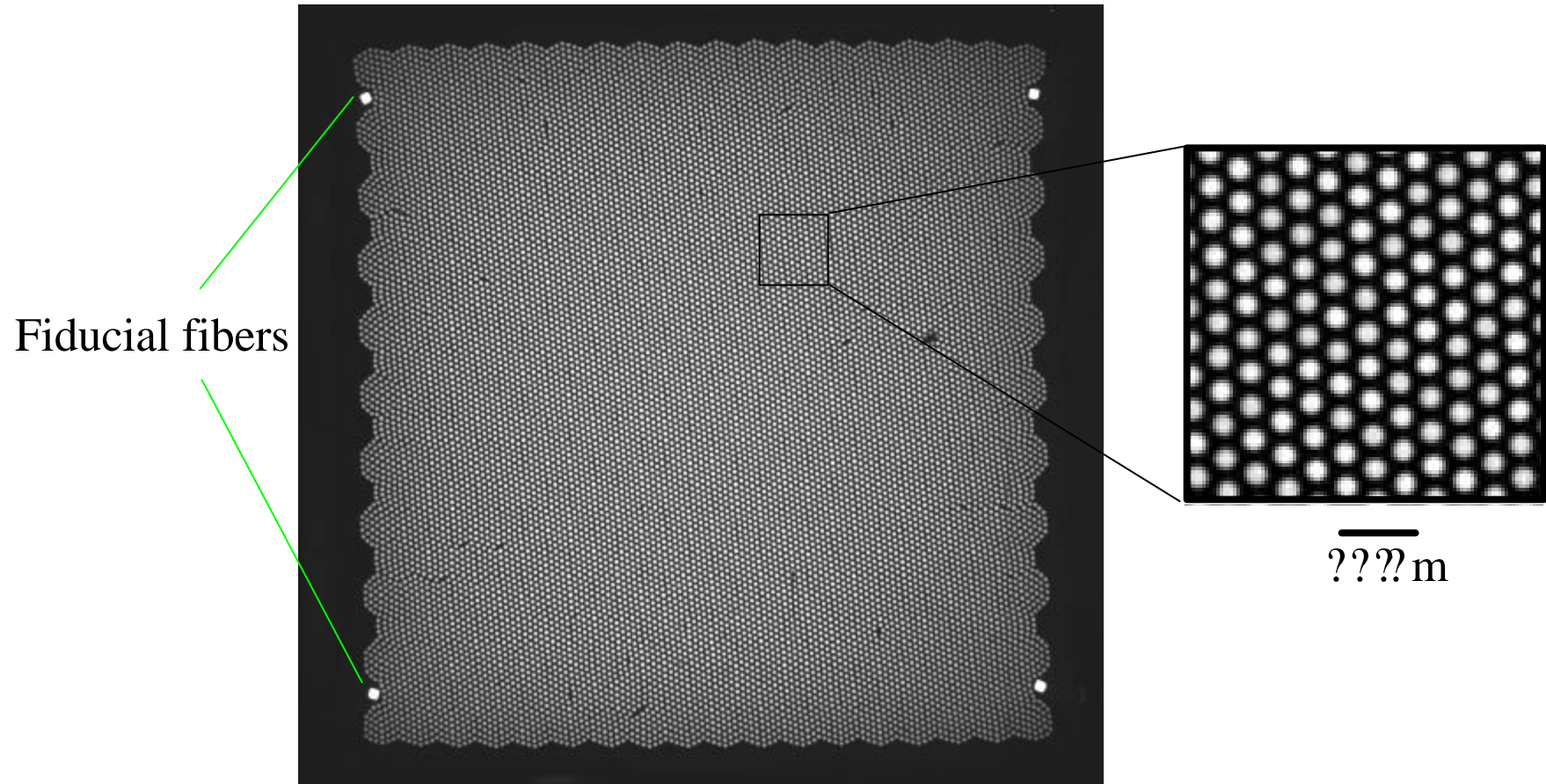


D.R. Walt, *Science*, 2000

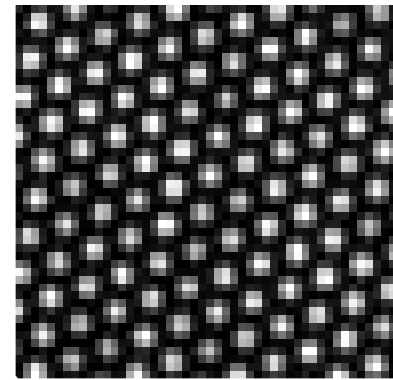
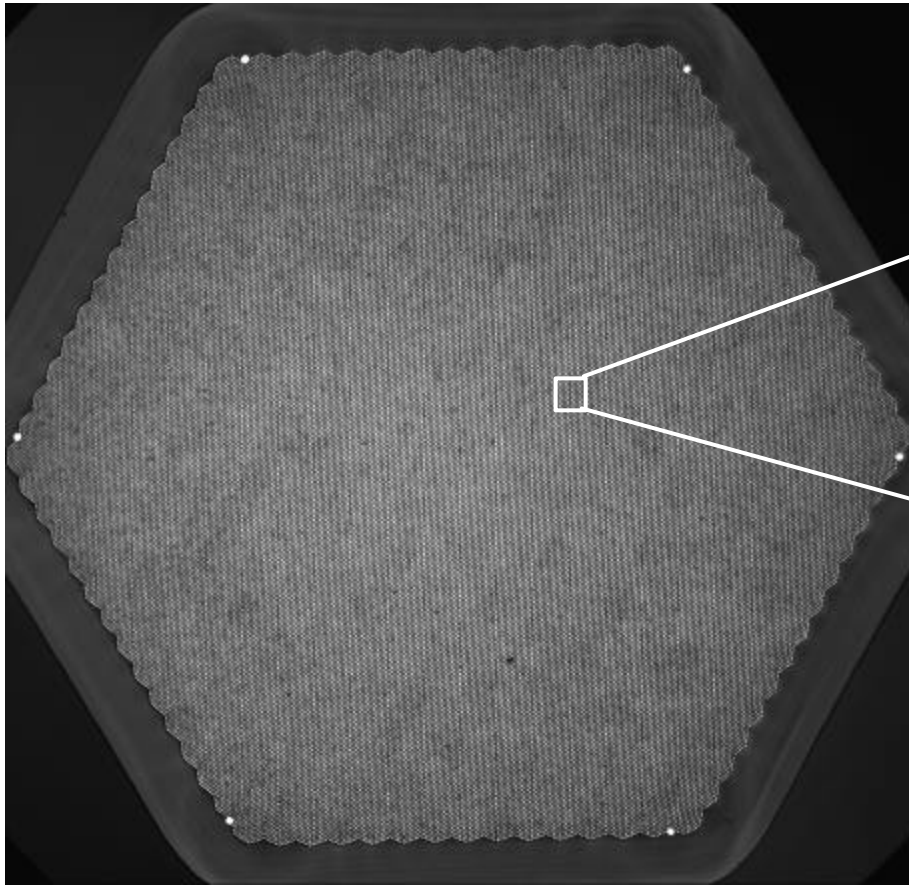
1K Fiber Bundle



13K Fiber Bundle

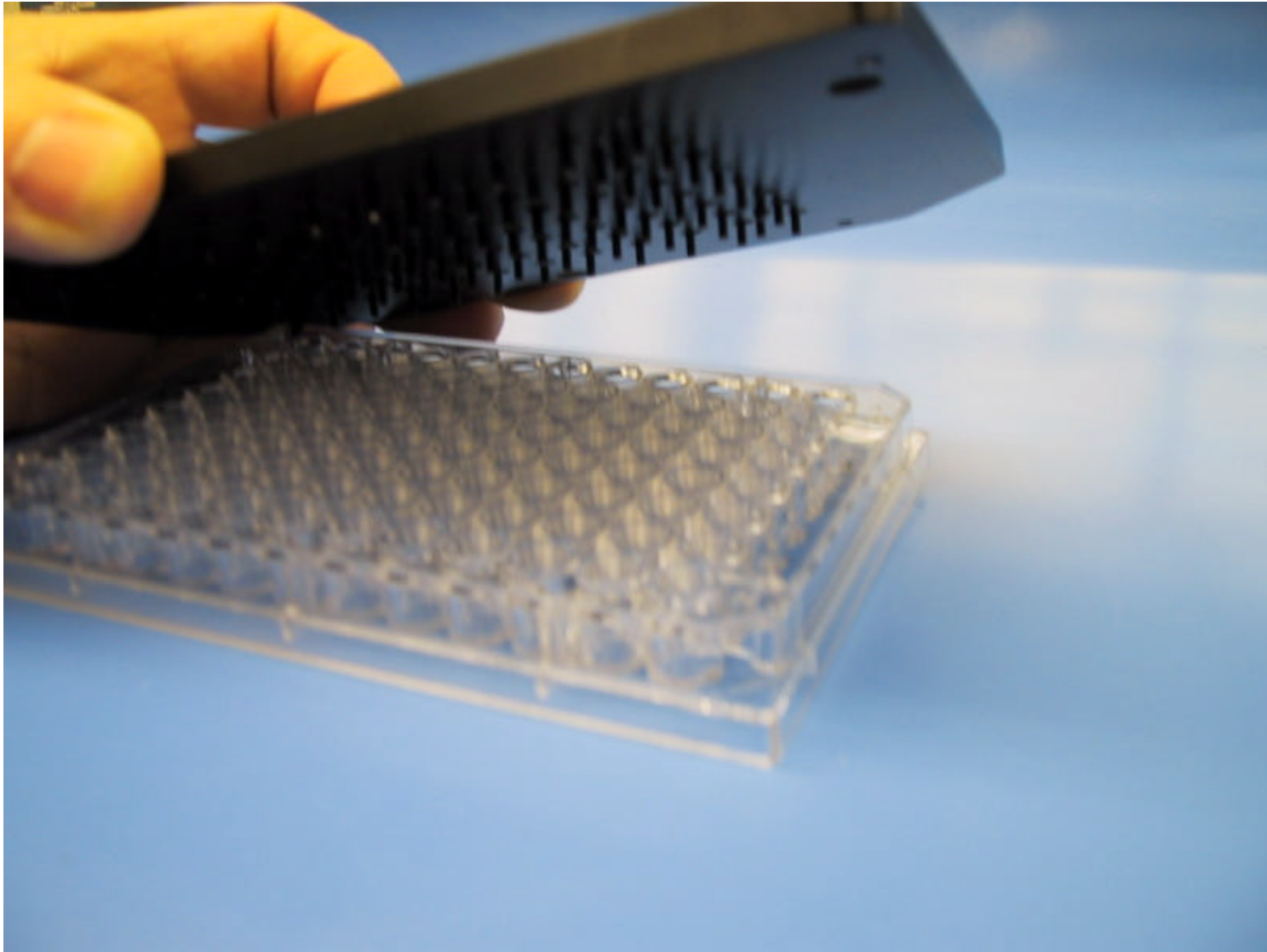


57K Fiber Bundle



??m

Array of Arrays™



Scalability of Technology

>2K

Unique Experiments
(with ~20-fold redundancy)

>32K

>3M

>750K

>190K

Bead Summing

| Concentration | Hybridization Time: No Summing | Hybridization Time: 100 Bead Summed |
|---------------|--------------------------------------|---|
| 100 pM | 10 minutes | 4 minutes |
| 10 pM | 30 minutes | 7 minutes |
| 100 fM | 4 hours | 20 minutes |
| 10 fM | 17 hours | 30 minutes |

Size and Concentration

| Volume | | | 1 M | 1 nM | 1 pM |
|-------------------------------|------|----------------------|--------------------|--------------------|--------------------|
| $(1 \text{ mm})^3$ | 1 L | 10^{-6} L | 6×10^{11} | 6×10^8 | 6×10^5 |
| $(100 \text{ }\mu\text{m})^3$ | 1 nL | 10^{-9} L | 6×10^8 | 6×10^5 | 6×10^2 |
| $(10 \text{ }\mu\text{m})^3$ | 1 pL | 10^{-12} L | 6×10^5 | 6×10^2 | 6×10^{-1} |
| $(1 \text{ }\mu\text{m})^3$ | 1 fL | 10^{-15} L | 6×10^2 | 6×10^{-1} | |
| $(0.1 \text{ }\mu\text{m})^3$ | 1 aL | 10^{-18} L | 6×10^{-1} | | |

Probe and Target Sequences for DNA Microarray Detection Limits

Probe

IL2 (interleuken-2) 5'-TA-CAA-GAA-TCC-CAA-ACT-CAC-CAG-3'

IL6 (interleuken-6) 5'-GT-TGG-GTC-AGG-GGT-GGT-TAT-T-3'

F508C 5'-TAG-GAA-ACA-CCA-CAG-ATG-ATA-3'

Target

IL2 (interleuken-2) 5'-CT-GGT-GAG-TTT-GGG-ATT-CTT-GTA-3'

IL6 (interleuken-6) 5'-AA-TAA-CCA-CCC-CTG-ACC-CAA-C-3'

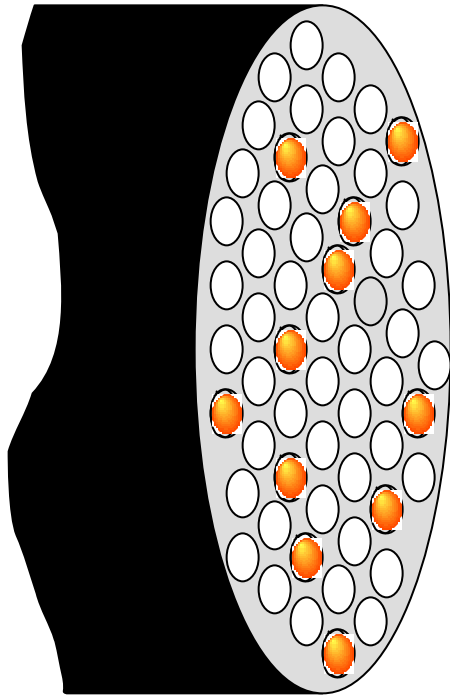
F508C 5'-TA-TCA-TCT-GTG-GTG-TTT-CCT-A-3'

DNA Minimum Hybridization Time with ICCD Camera

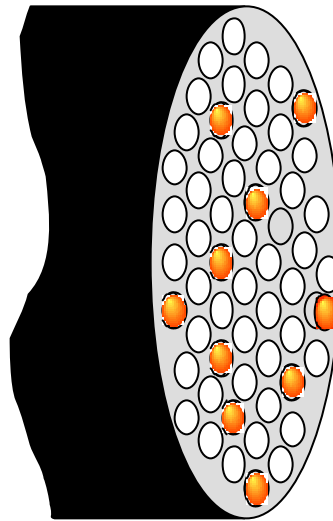
| DNA Concentration | Hybridization Time (min) |
|-------------------|--------------------------|
| 1 pM | 10 |
| 100 fM | 20 |
| 10 fM | 30 |
| 1 fM | 60 |

Detection Limit Problem

Multiple beads provides a signal averaging benefit.



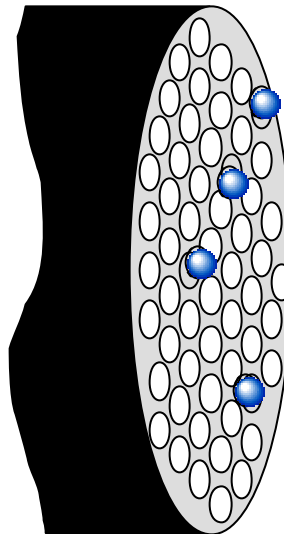
S/N increases by \sqrt{n}



$\frac{1000 \text{ target molecules}}{10 \text{ beads}}$

$=100 \text{ target molecules/bead}$

Fewer beads provide more target molecule numbers per bead.



$\frac{1000 \text{ target molecules}}{4 \text{ beads}}$

$=250 \text{ target molecules/bead}$

Multiplexed Array Sensitivity and Selectivity with 1 fM IL2 Target Solutions

IL2 Target - 1 fM concentration - 12 hour hybridization time

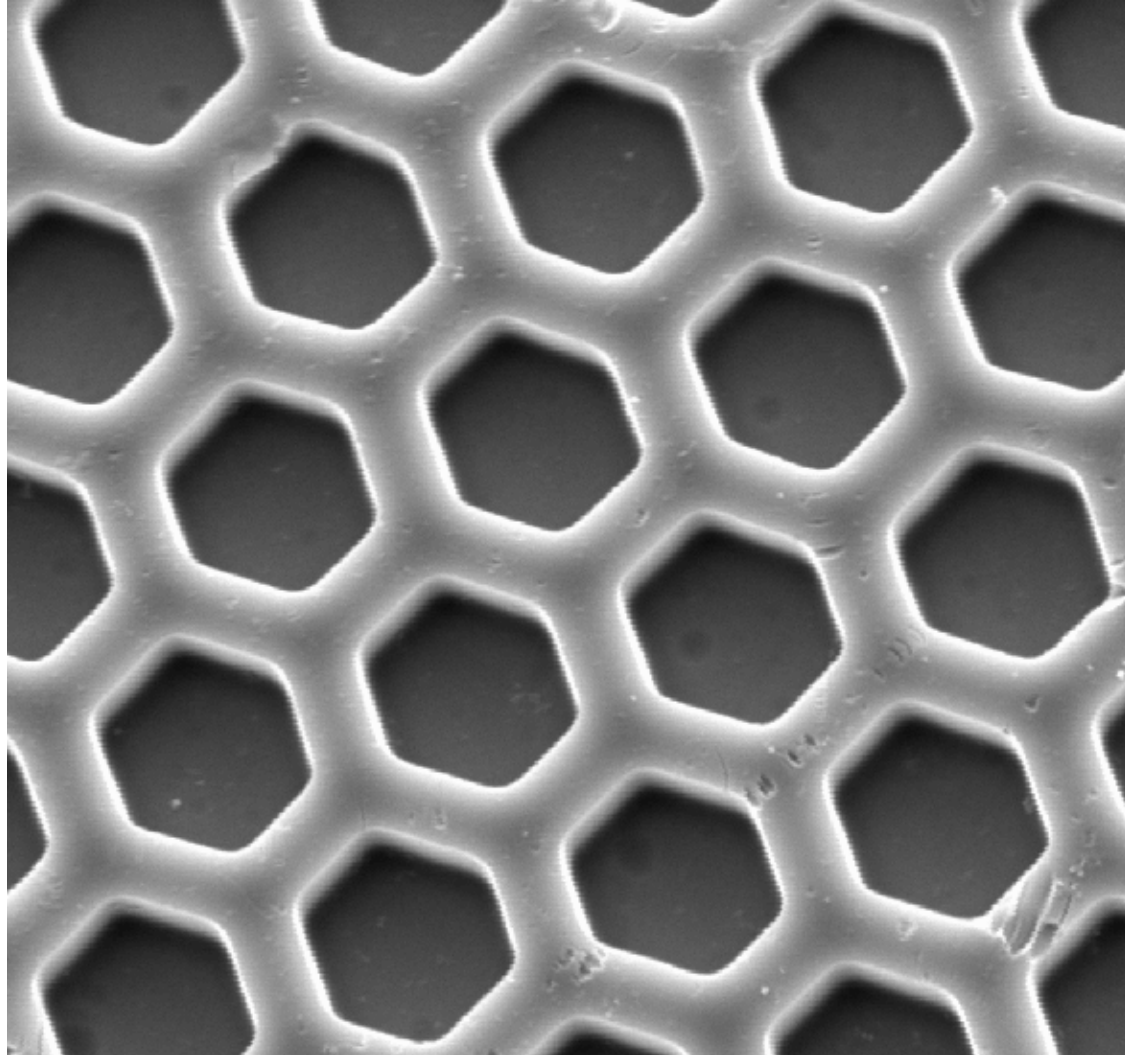
| Target/Probe | | Mean background \pm s.d | Hybridization \pm s.d. | Signal \pm s.d |
|---------------------|-------|---|--|------------------------------------|
| F508 | F508C | 530.43 ± 1.8 | 550.17 ± 7.5 | $\{19.74\} \pm 7.7$ |
| | IL2 | 563.99 ± 7.7 | 677.08 ± 8.1 | 113.09 ± 11 |
| | IL6 | 445.99 ± 3.9 | 449.16 ± 1.4 | $\{3.17\} \pm 4.1$ |
| IL2 | F508C | 439.64 ± 3.5 | 443.34 ± 5.6 | $\{3.70\} \pm 6.6$ |
| | IL2 | 432.52 ± 5.6 | 503.31 ± 6.6 | 70.79 ± 8.7 |
| | IL6 | 431.11 ± 2.1 | 432.13 ± 2.8 | $\{1.02\} \pm 3.5$ |
| IL6 | F508C | 454.84 ± 3.6 | 465.82 ± 1.4 | $\{10.98\} \pm 3.8$ |
| | IL2 | 429.42 ± 0.92 | 517.38 ± 2.6 | 87.96 ± 2.8 |
| | IL6 | 459.81 ± 3.0 | 467.82 ± 5.3 | $\{8.01\} \pm 6.1$ |

Microsphere Array Sensitivity and Selectivity with 100 aM IL2 Target Solutions

IL2 Target - 100 aM concentration - 12 hour hybridization time

| Probe/Target | | Mean background \pm s.d | Hybridization \pm s.d. | Signal \pm s.d |
|--------------|-------|---------------------------|--------------------------|---------------------|
| IL2 | F508C | 386.97 ± 3.2 | 387.98 ± 1.4 | $\{1.01\} \pm 3.5$ |
| | IL2 | 378.55 ± 2.3 | 394.00 ± 3.7 | 15.32 ± 4.4 |
| | IL6 | 382.80 ± 6.3 | 393.81 ± 6.1 | $\{11.01\} \pm 7.1$ |
| IL2 | F508C | 268.66 ± 2.3 | 274.22 ± 8.5 | $\{5.56\} \pm 8.8$ |
| | IL2 | 297.73 ± 2.3 | 310.02 ± 2.3 | 12.29 ± 3.2 |
| | IL6 | 247.59 ± 2.7 | 248.70 ± 6.9 | $\{1.11\} \pm 7.4$ |
| IL2 | F508C | 410.73 ± 2.6 | 413.63 ± 2.6 | $\{2.90\} \pm 2.9$ |
| | IL2 | 410.69 ± 2.7 | 455.26 ± 6.5 | 44.57 ± 7.0 |
| | IL6 | 390.24 ± 7.4 | 392.88 ± 2.8 | $\{2.64\} \pm 7.9$ |

SEM of a Microwell Array

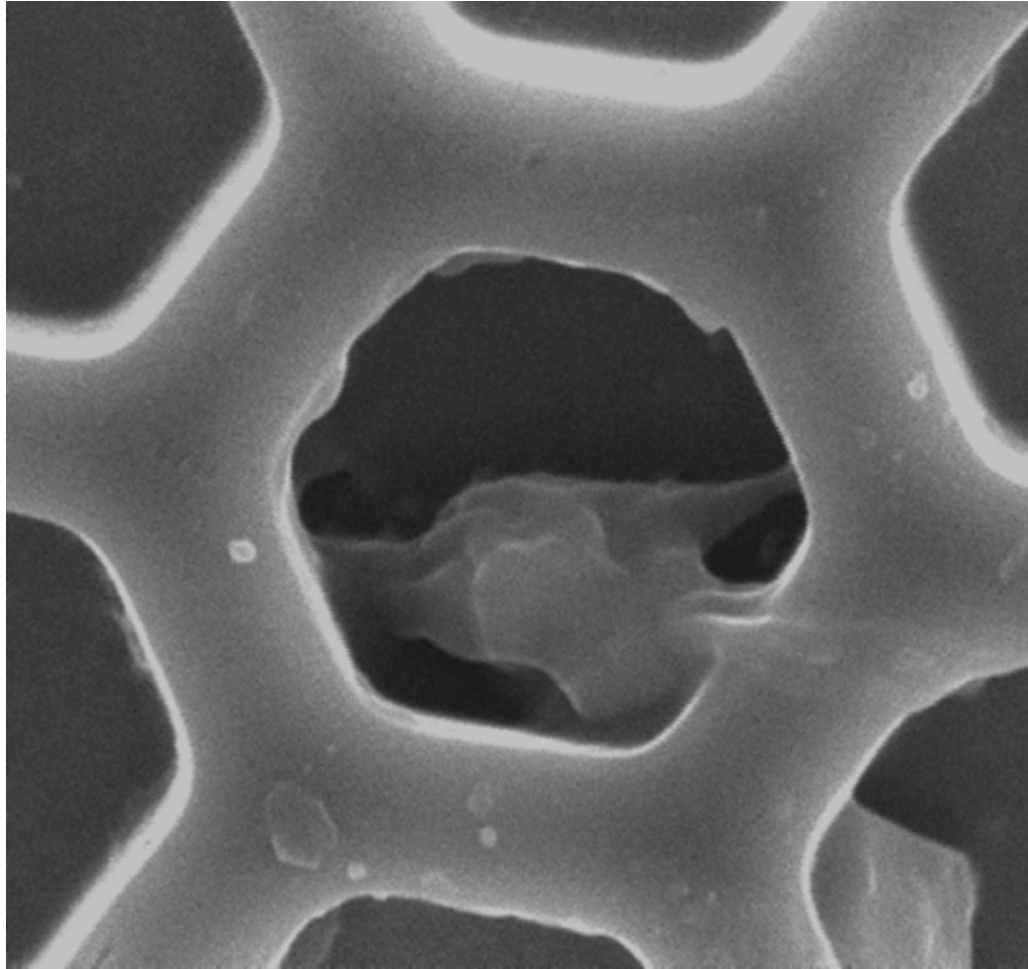


7 μ m well diameter

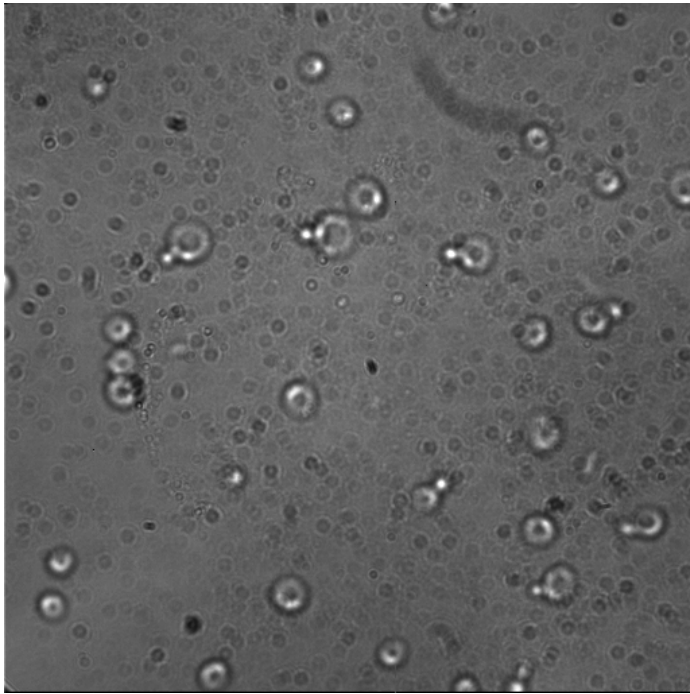
~3 μ m well depth

~90 fL well volume

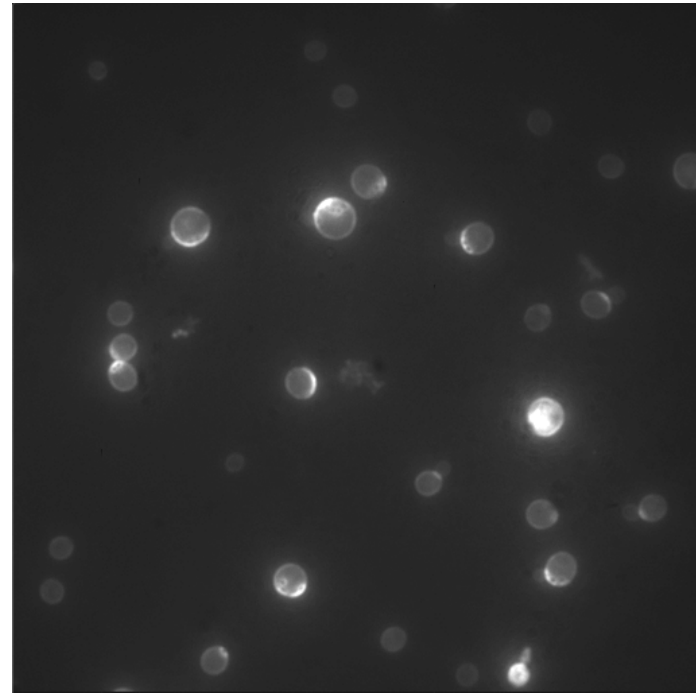
Single NIH 3T3 Mouse Fibroblast Cell in a Fiber-optic Microwell



Single Yeast (*Saccharomyces cerevisiae*) Cells Array

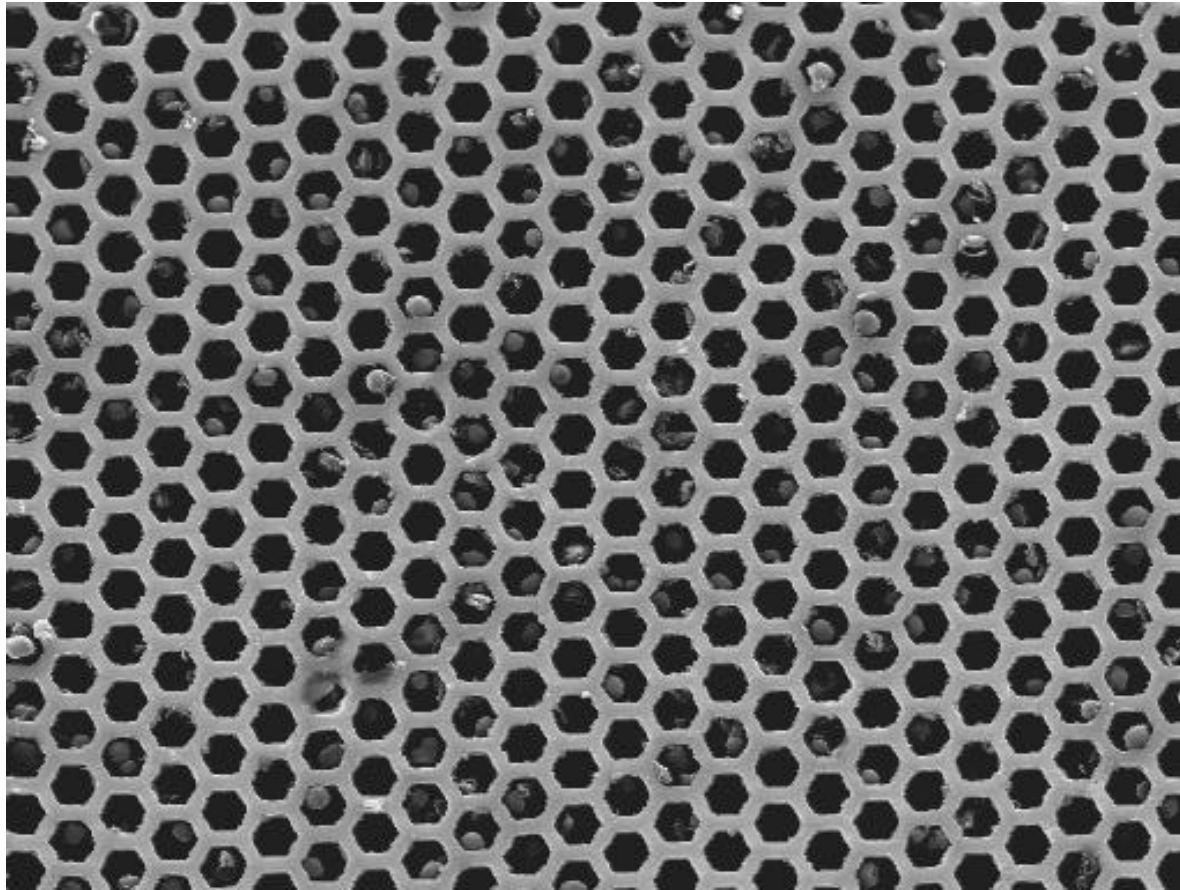


White light

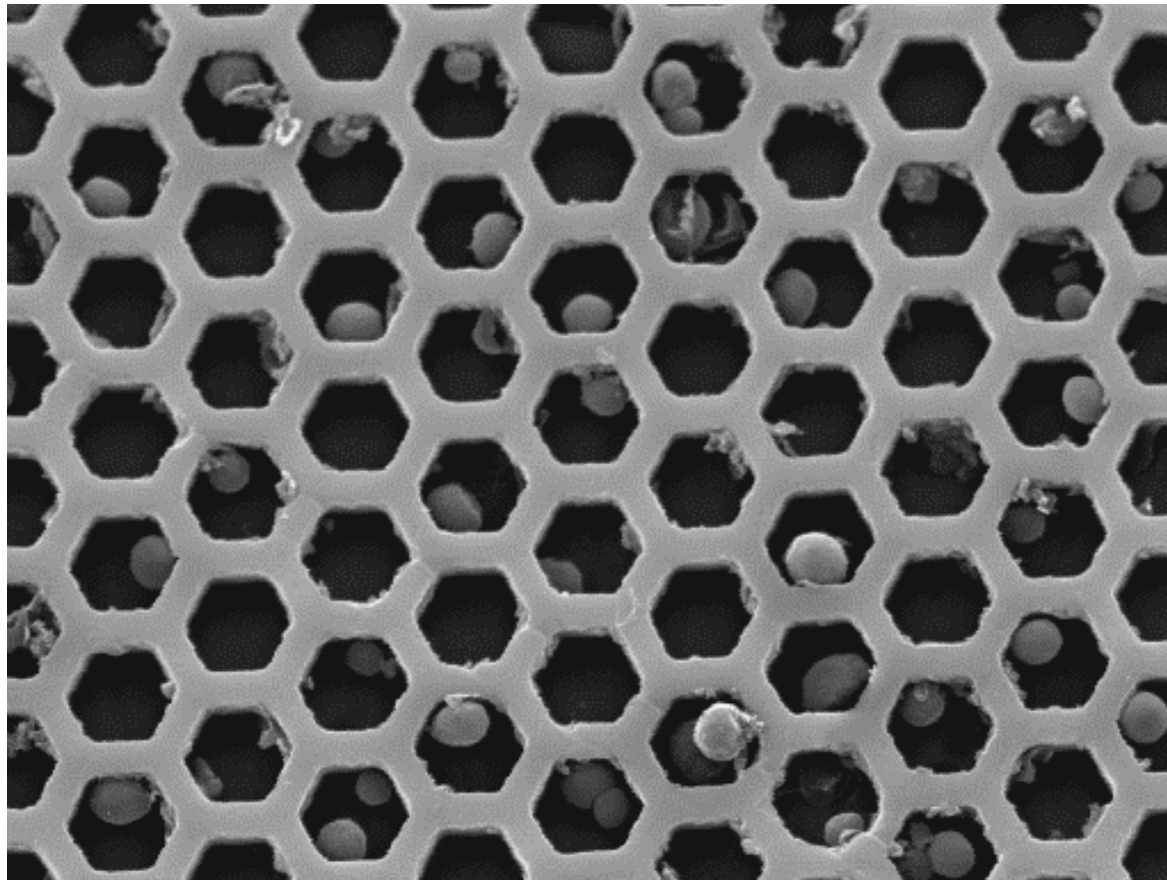


Calcofluor White 360/440

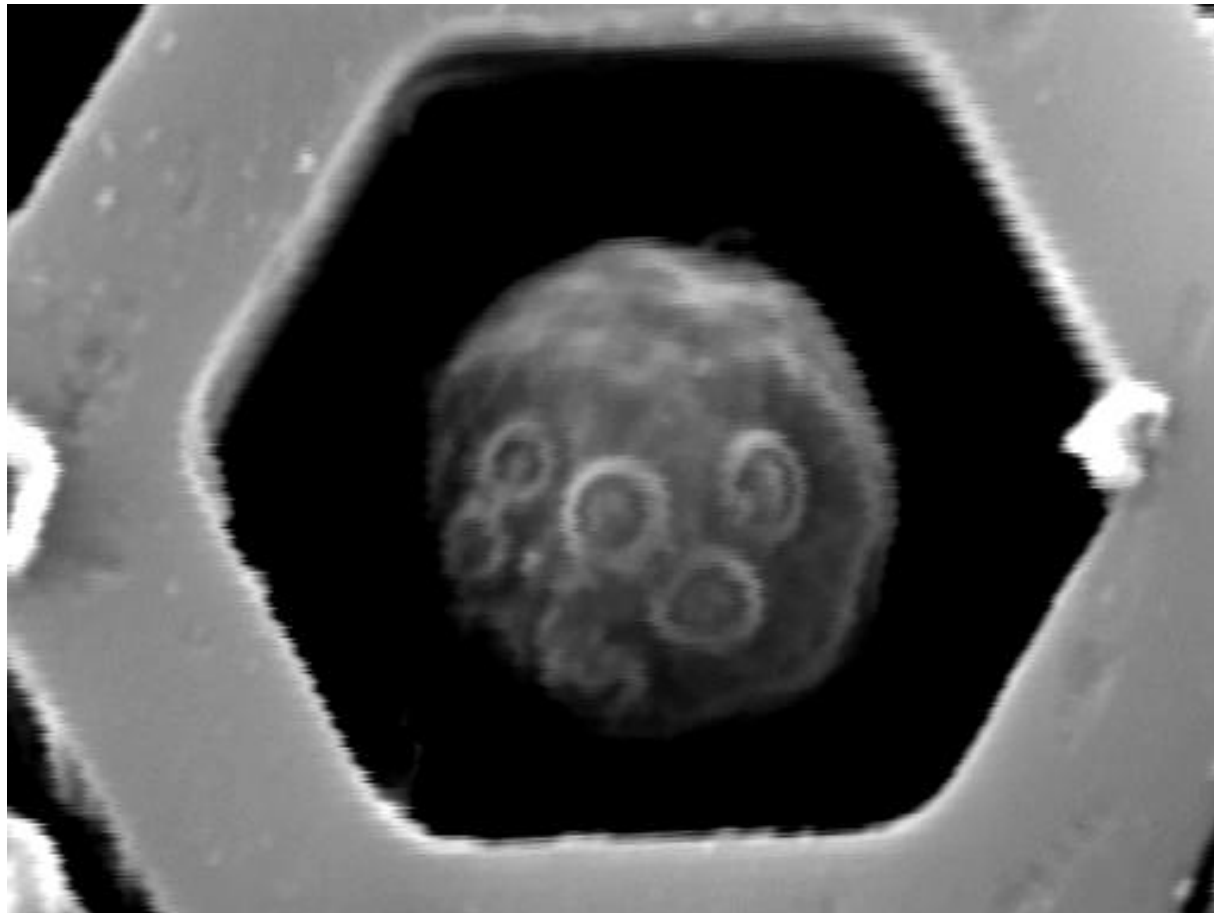
SEM images of Single Yeast Cells on the Microwells array



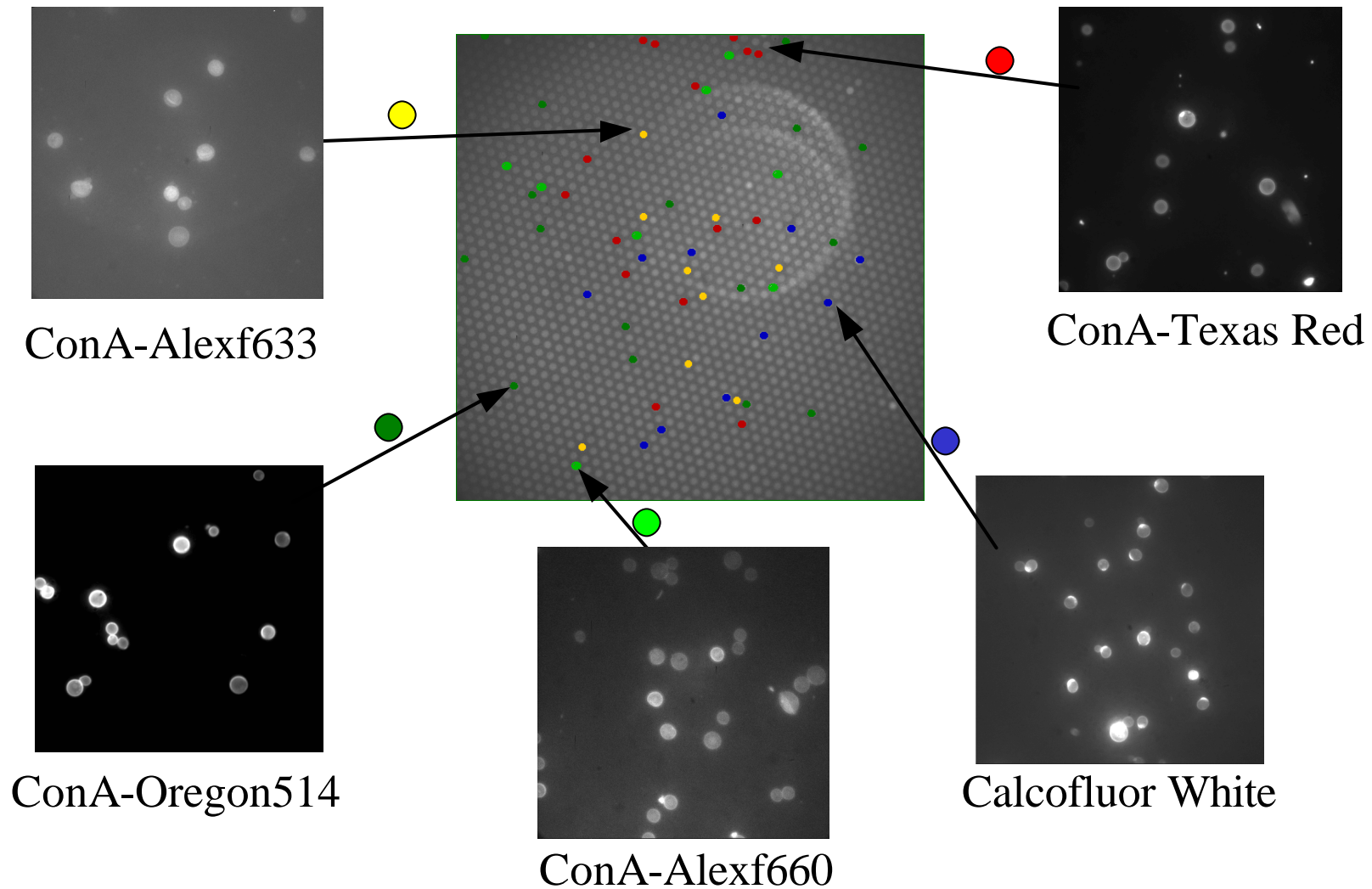
SEM images of Single Yeast Cells on the Microwells array



SEM images of Single Yeast Cells on the Microwells array

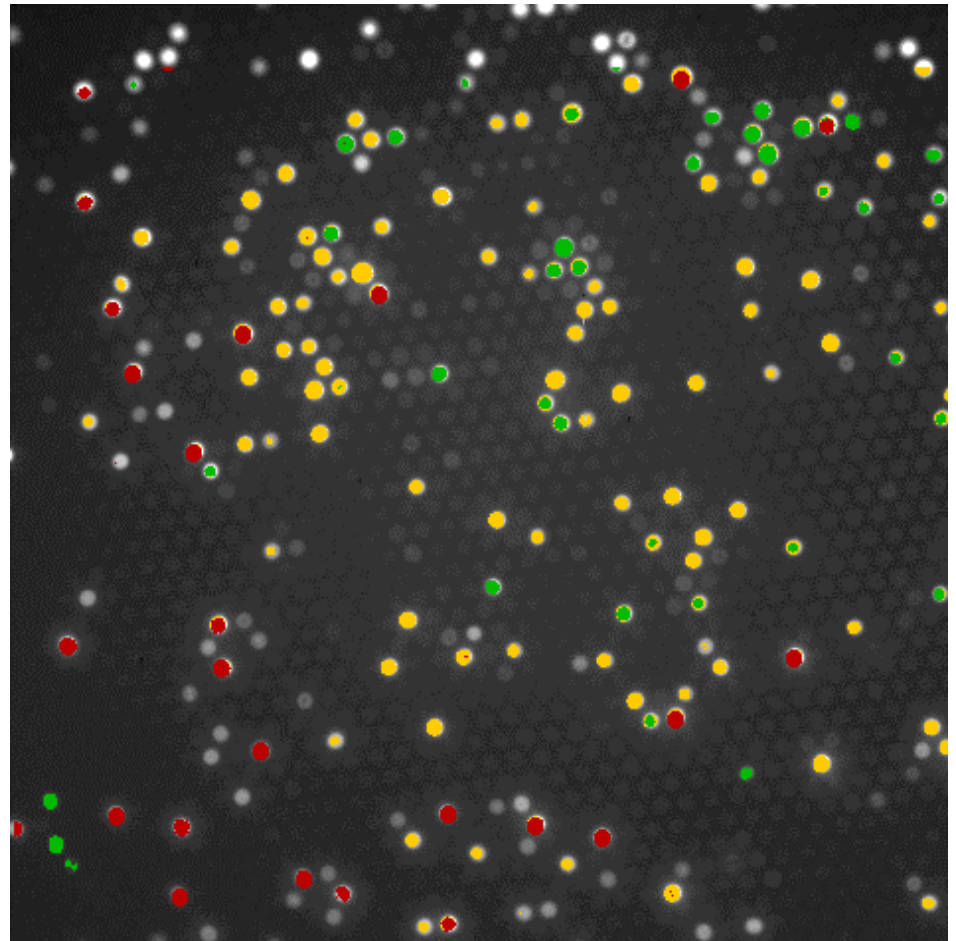


Encoded Yeast Cells on the Fiber Array



pH Measurement of Single Yeast Cells Microenvironment in the Array

- Concanavalin A-FITC
- Concanavalin A-FITC
+
Concanavalin A-Alexa fluor 660
- Concanavalin A-FITC
+
Concanavalin A-Texas Red



Smarter Sensors- Anticipatory

Is it bad?

What does it resemble?

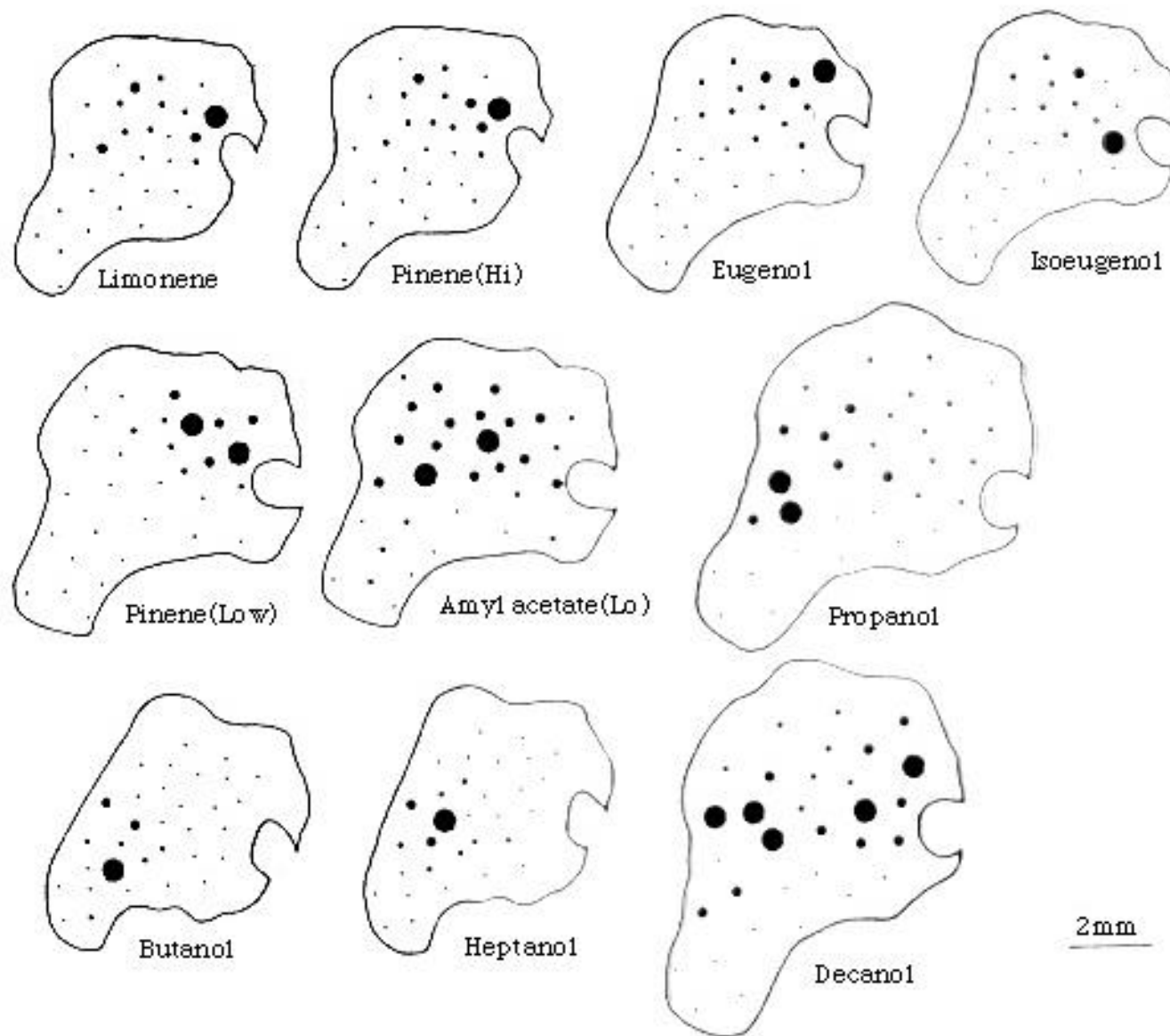
What will it do?

e.g. GI, neurotoxic, etc.

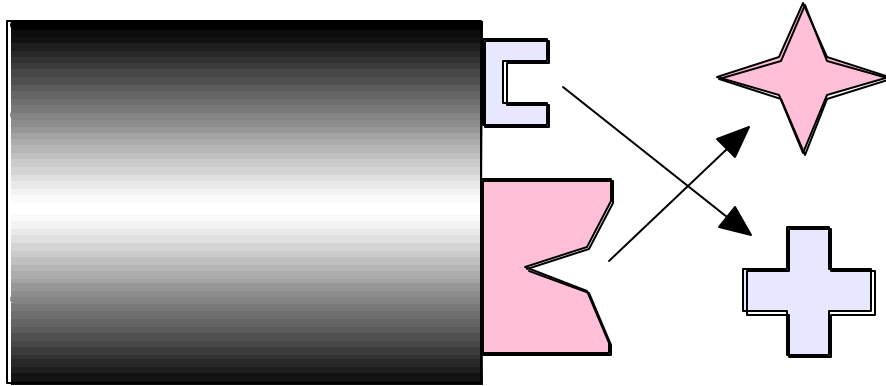
“common virulence mechanisms”



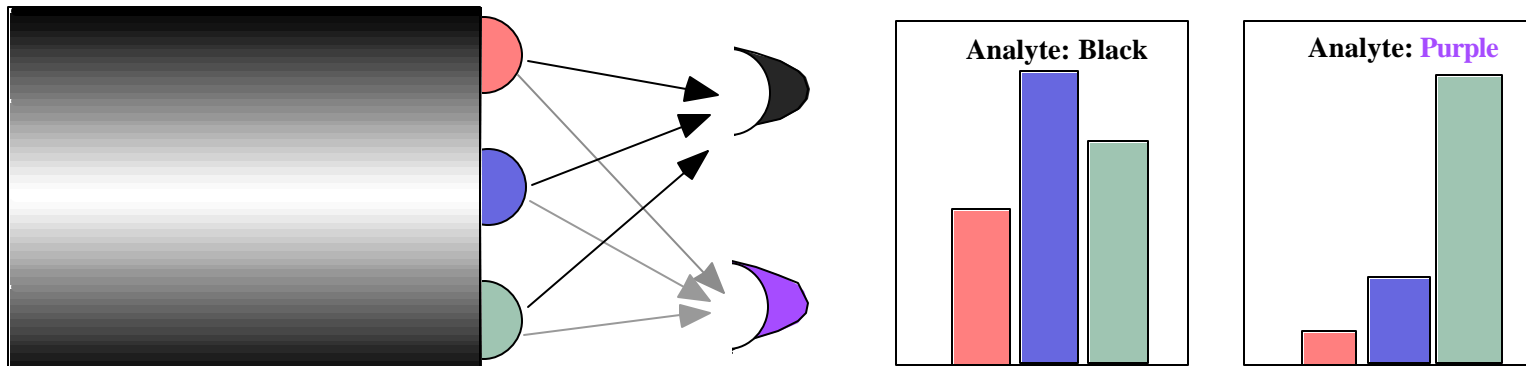
surrogates



Sensor Design

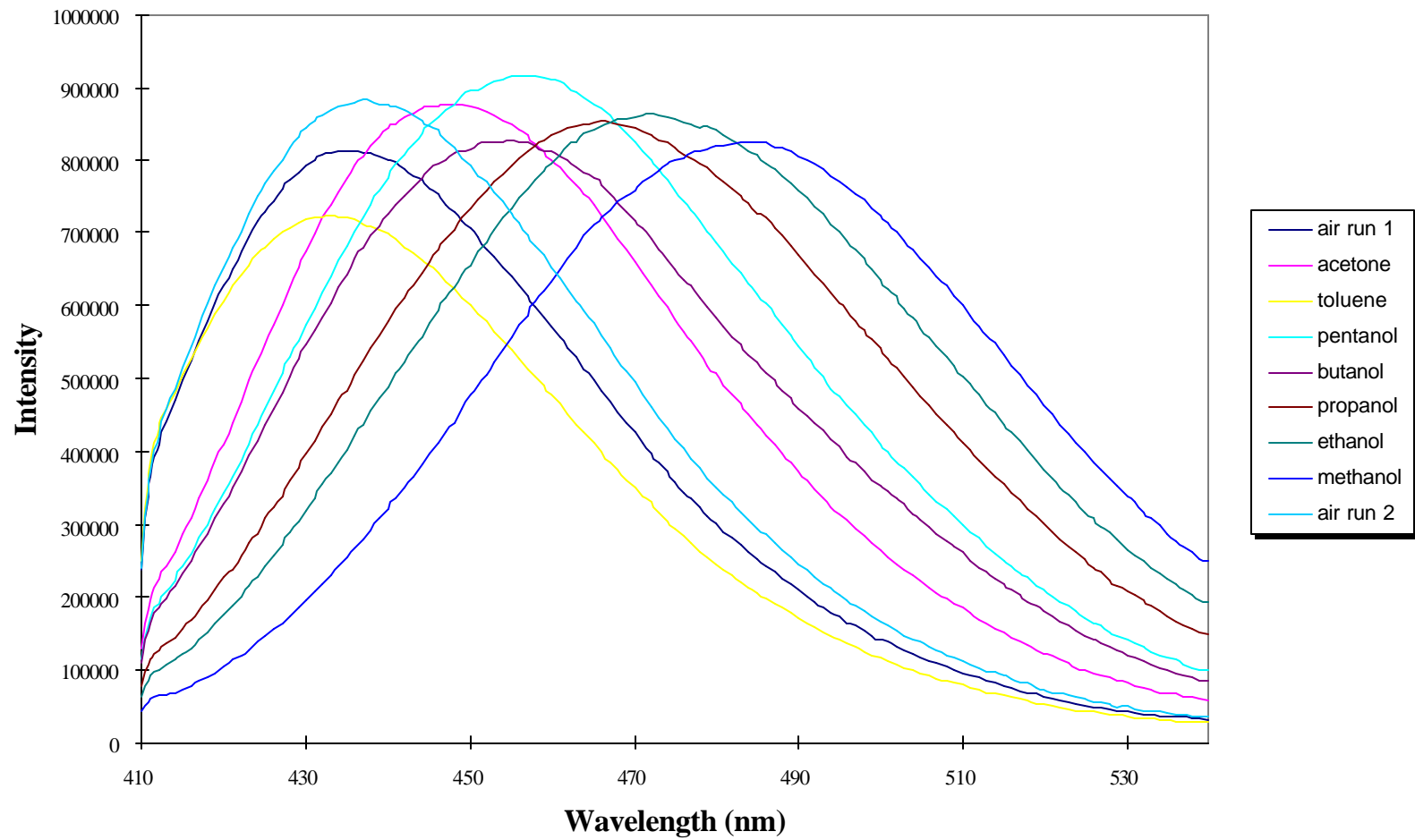


A) Lock-and-key Sensor

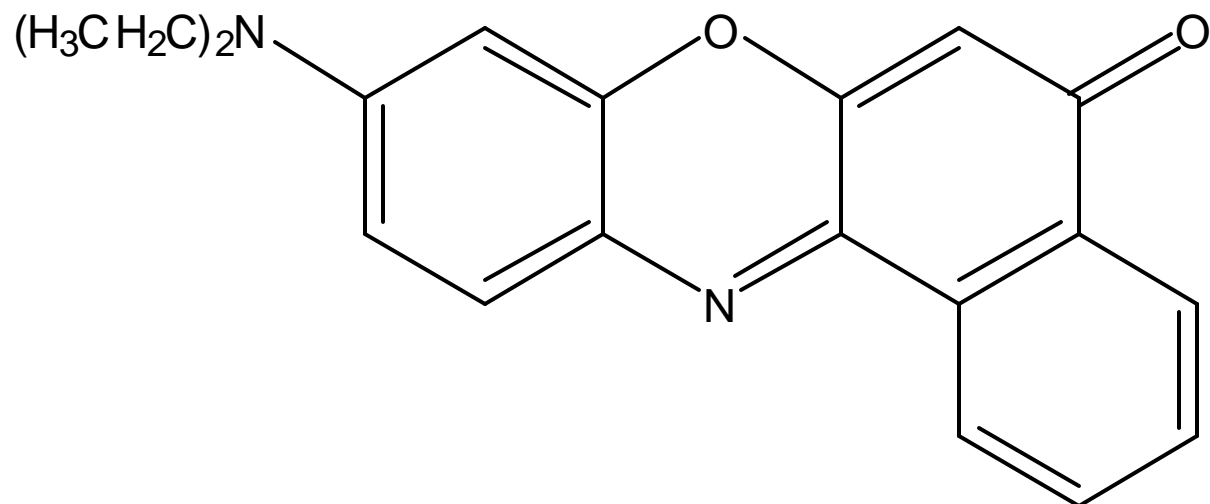


B) Cross-reactive Sensor

Solvatochromic Effect

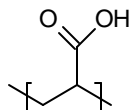


Nile Red

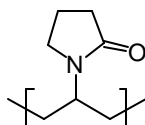


Role of Polymer Polarity

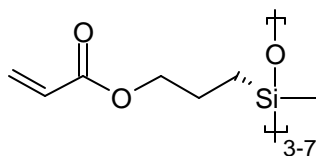
poly(acrylic acid), **PAA**



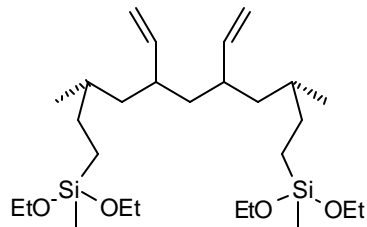
poly(N-vinyl pyrrolidone), **PVP**



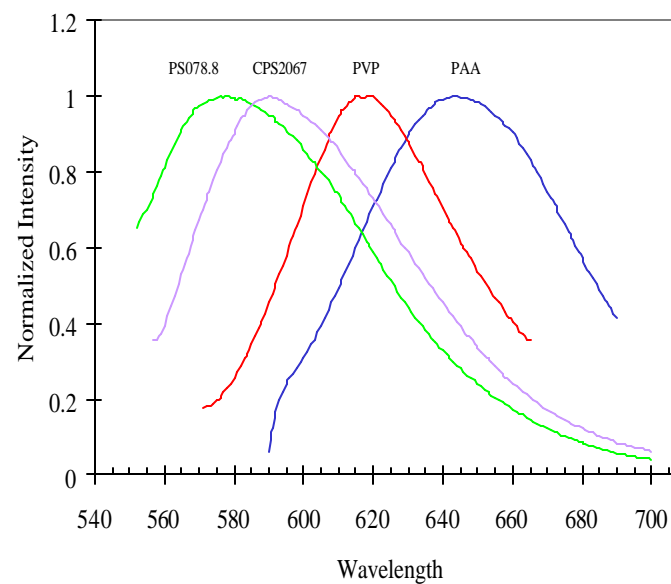
acryloxypropylmethyl-
cyclosiloxane, **CPS2067**



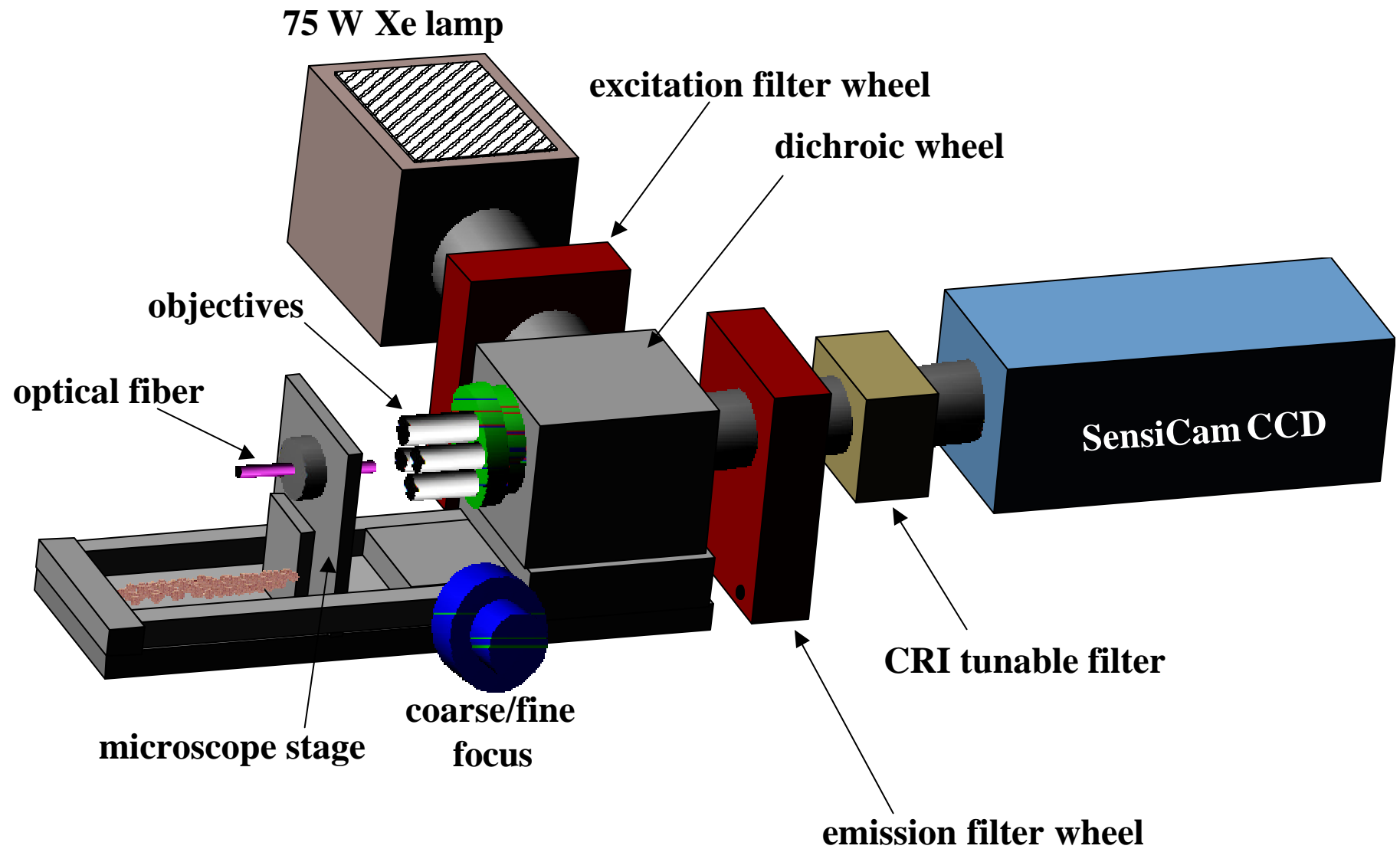
diethoxymethylsilyl-modified
polybutadiene, **PS078.8**



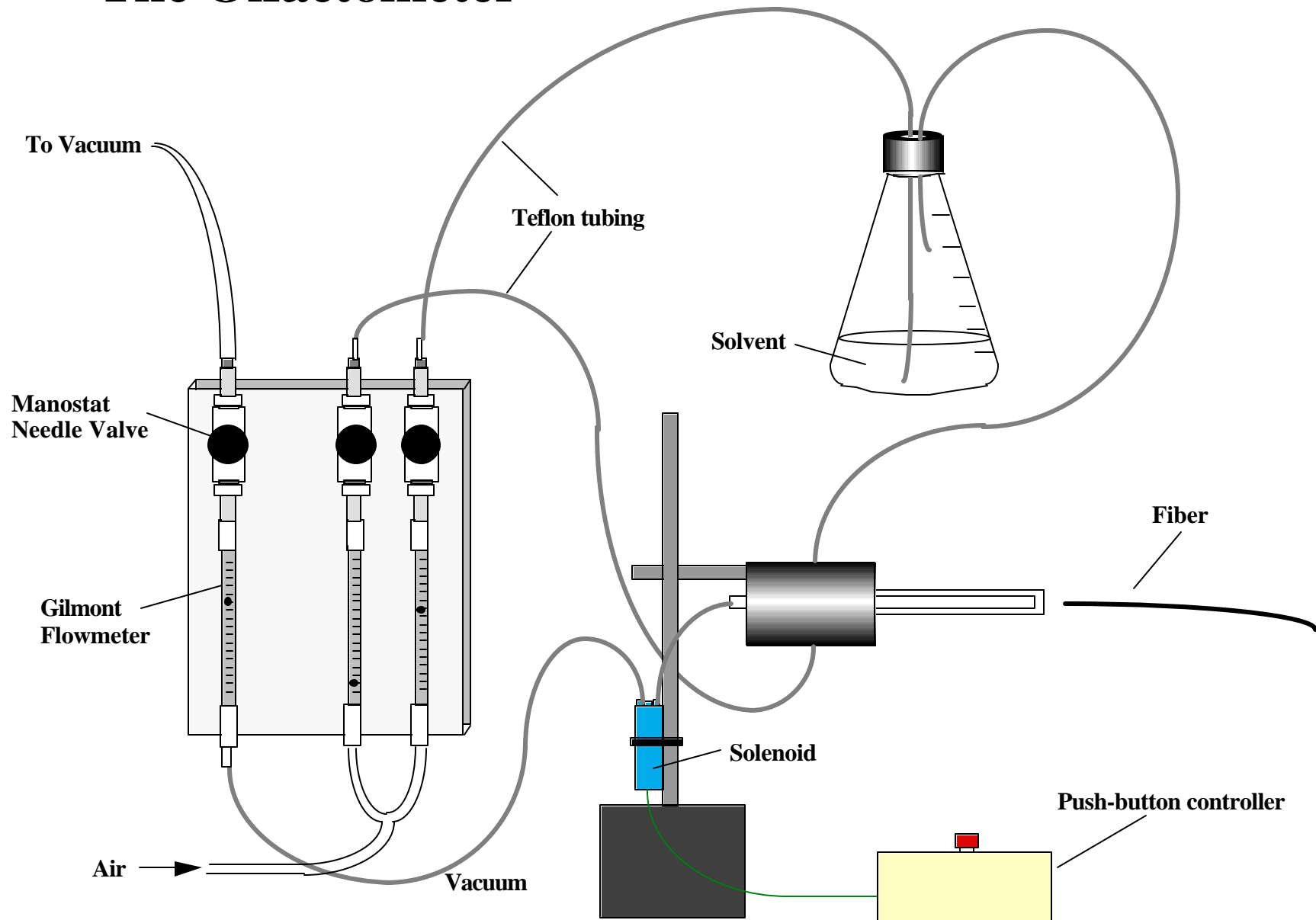
Decreasing
polarity



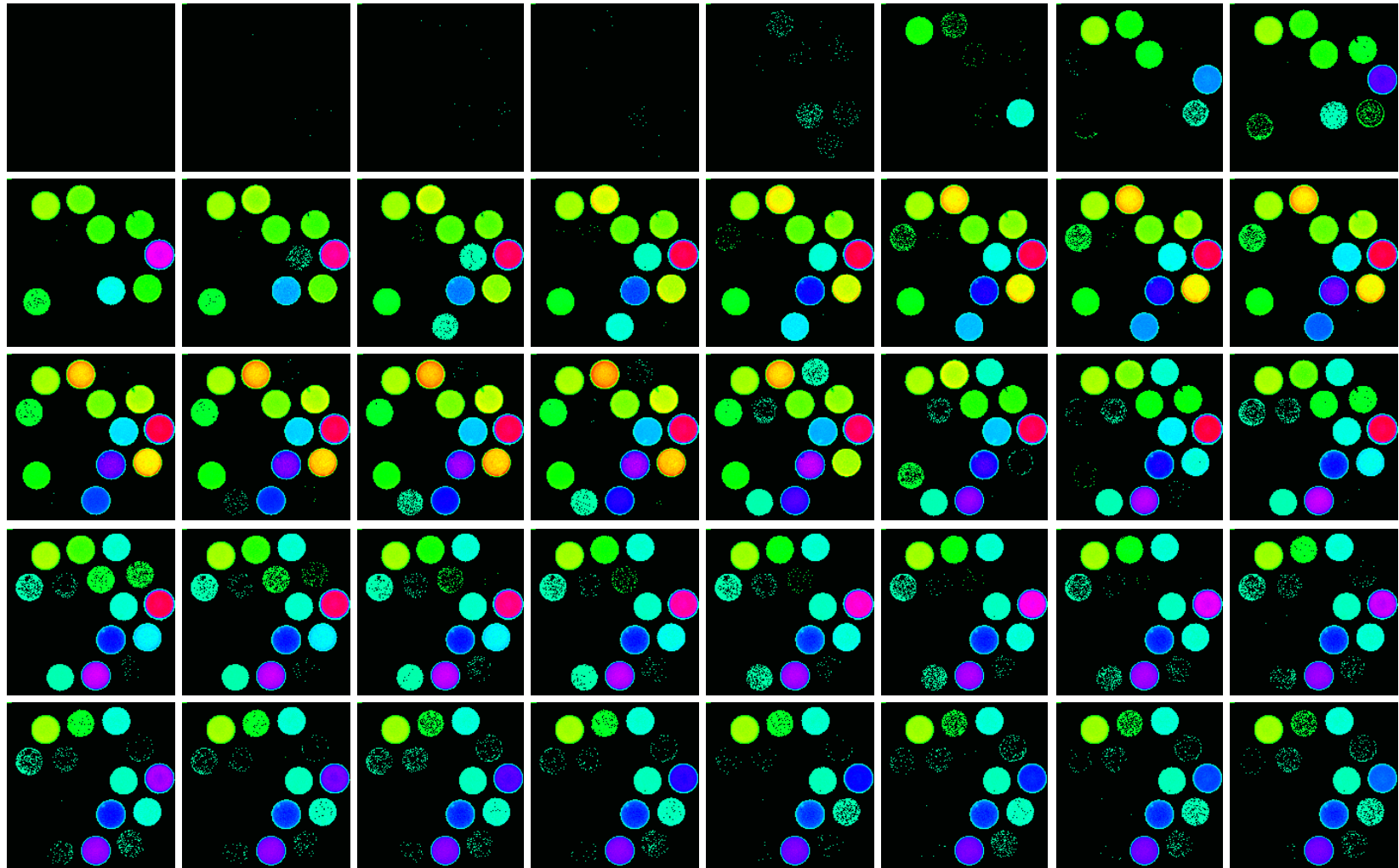
CCD-based imaging system



The Olfactometer — J. Kauer

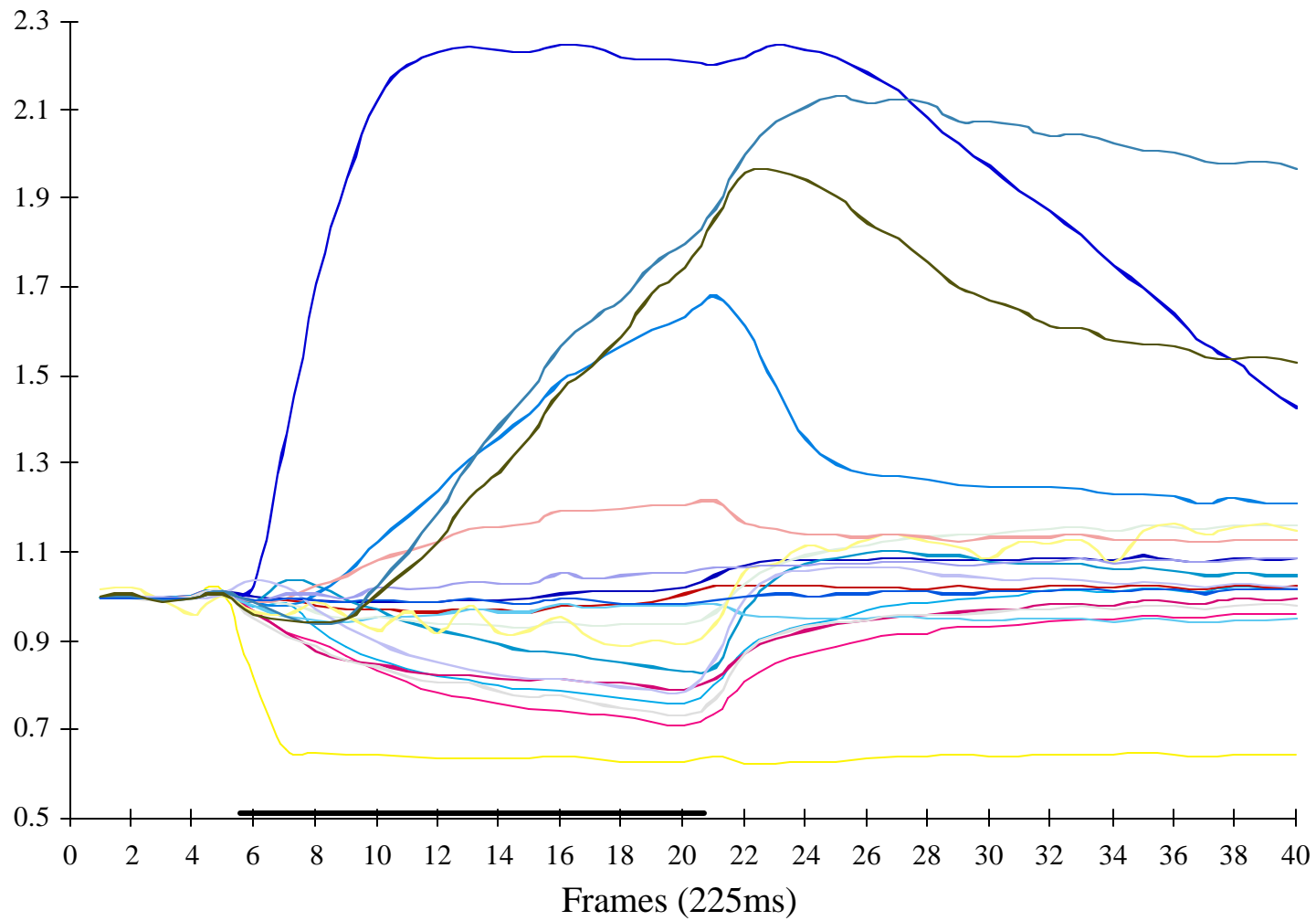


Sensor Array Response to Benzene Vapor Pulse



Dickinson, T. A., *et al.* (1996) *Nature*, 382: 697-700.

Temporal Plots from 19-Fiber Sensor Array Response to Benzene Vapor Pulse



Classification Results

Learning Vector Quantization Approach

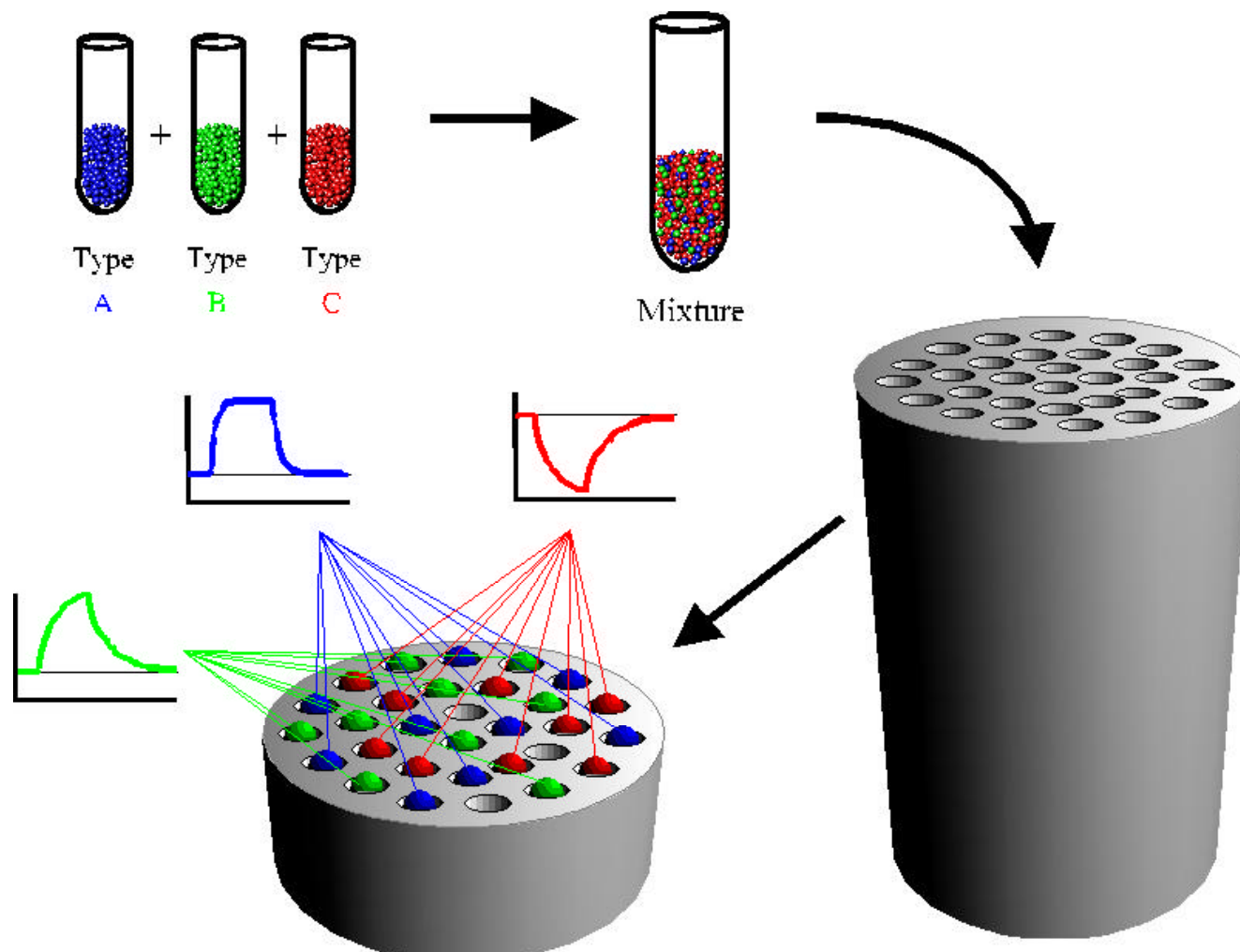
True Identity

Network Output

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1 | 24 | | | | | | | | | | | | | | | | | | | |
| 2 | | 23 | | | | | | | | | | 1 | | | | | | | | |
| 3 | | | 20 | | | | | | | 3 | | | 1 | | | | | | | |
| 4 | | | 1 | 19 | | | | | 1 | | | | | 1 | | | | 2 | | |
| 5 | | | 1 | | 17 | 4 | | | 2 | | | | | | | | | | | |
| 6 | | | | 1 | 2 | 19 | | | | | 1 | | | | 1 | | | | | |
| 7 | | | | | | | 18 | 4 | | 1 | | | 1 | | | | | | | |
| 8 | | | | | | | 2 | 21 | | | | | 1 | | | | | | | |
| 9 | | | | 1 | | | | | 23 | | | | | | | | | | | |
| 10 | | | 3 | | | | | | | 19 | | | 1 | | | 1 | | | | |
| 11 | | | | | | | | | | | 21 | | | | | 1 | | | 2 | |
| 12 | | | | | | | | | | | | 23 | | | | | | 1 | | |
| 13 | | | | | | | | | 1 | | | | 23 | | | | | | | |
| 14 | | | | | | | | 1 | | | | | | 20 | | | 2 | | 1 | |
| 15 | | 1 | | | | | | | | | | | | | 23 | | | | | |
| 16 | | | | | | | | | | | | | | | | 24 | | | | |
| 17 | | | | | | | | | | | | | | | | | 24 | | | |
| 18 | | | | | | | | | | | | | | | | | | 24 | | |
| 19 | | | | | | | | | | | 4 | | | | | | | | 20 | |
| 20 | | 1 | | | | | | | | | | | | | | | | | | 23 |

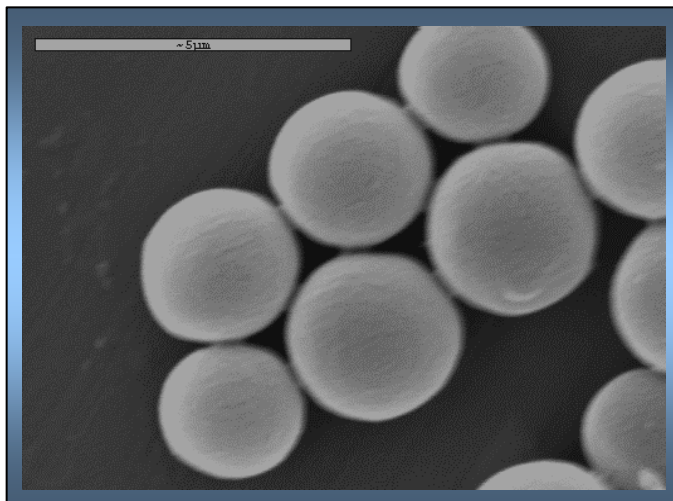
- 1) Acetone
- 2) Butyl Acetate
- 3) Beauty
- 4) Camphor
- 5) Carvone -
- 6) Carvone +
- 7) Chloroform
- 8) Dichloroethane
- 9) DMSO
- 10) Drakkar Noir
- 11) Water
- 12) Heptane
- 13) Isopropanol
- 14) Indole
- 15) Mercaptoethanol
- 16) Methanol
- 17) Propanol
- 18) Propionic Acid
- 19) Pseudoexplosive
- 20) Toluene

SENSOR ARRAYS are Assembled 'Randomly' in ONE Fabrication Step

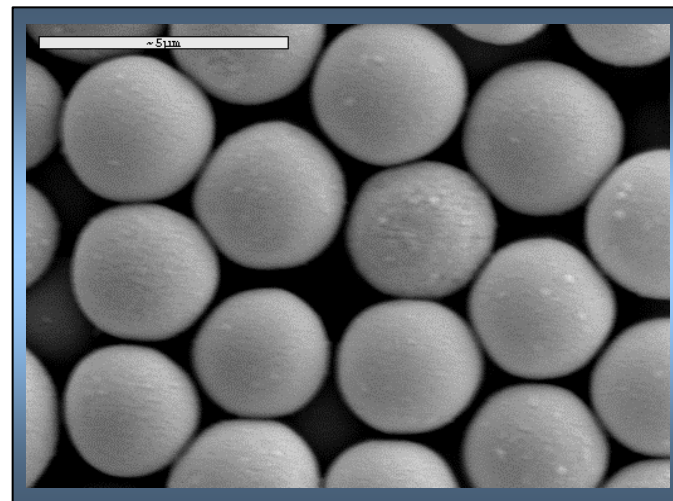


NOTE: the Sensor Array is a 'Self-Encoding' Bead Array (SEBA). Billions of Sensors are Fabricated at Once.

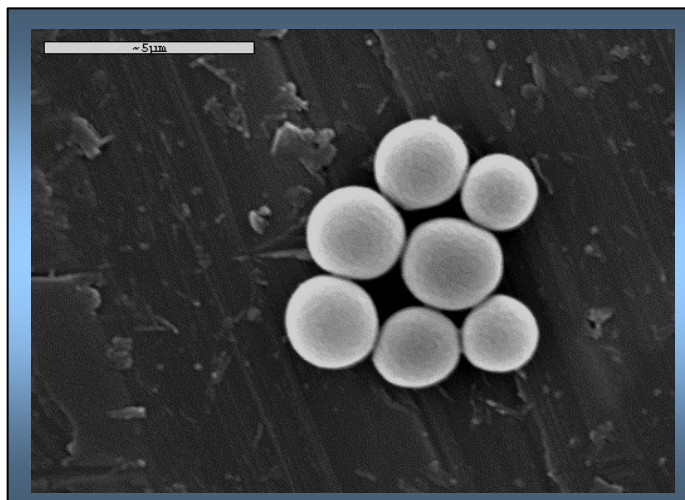
Hollow Poly(benzyl methacrylate) Spheres



3.5 h polymerization



6.5 h polymerization

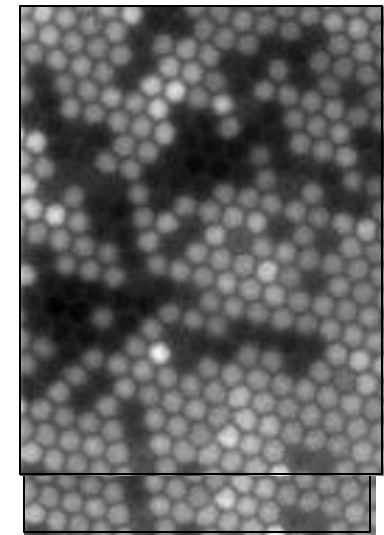
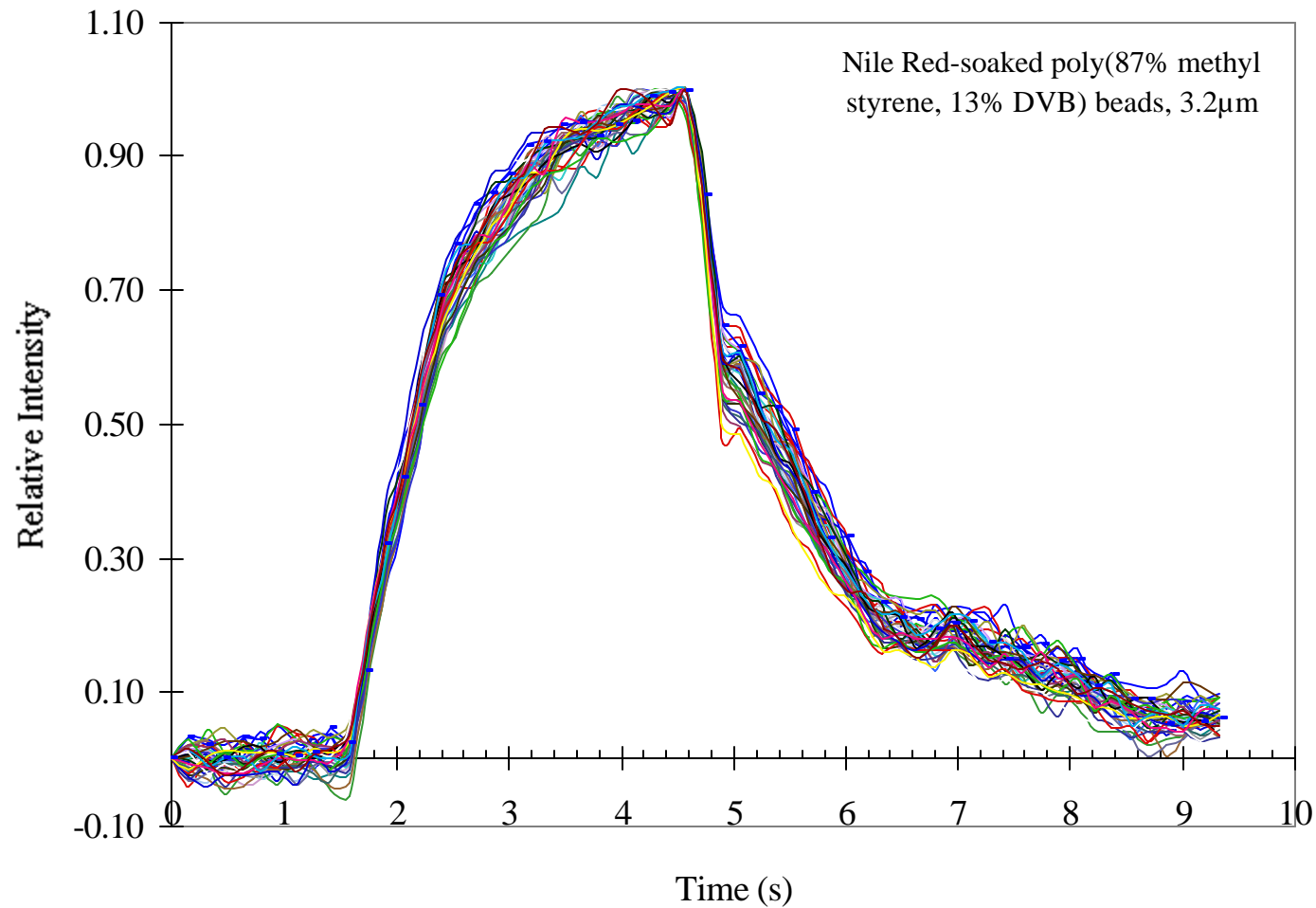


14h polymerization

Chem. Mater. 2000

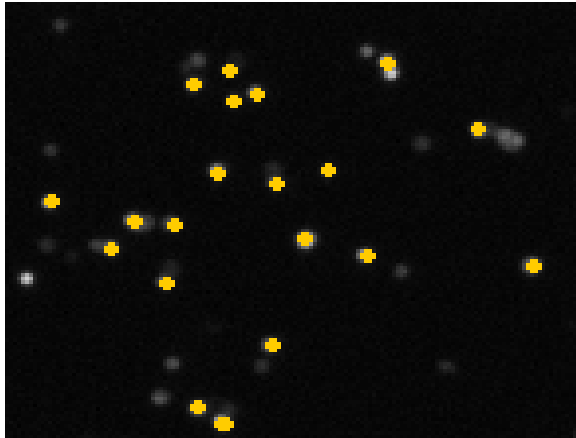
Nile Red/PolyMethylStyrene Beads in Wells:

Response of 40 beads to methanol pulse

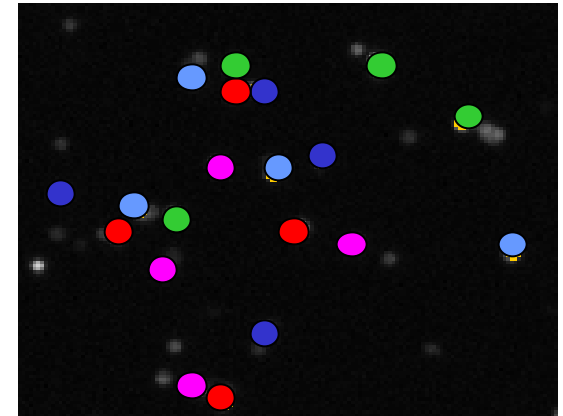


bright = wells with beads
dark = empty wells

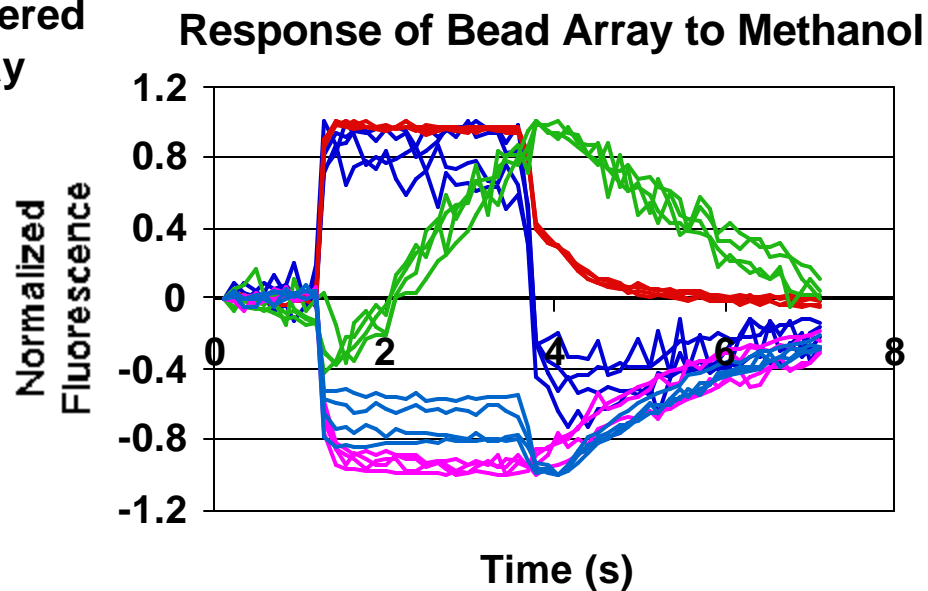
Sensor Registration Problem



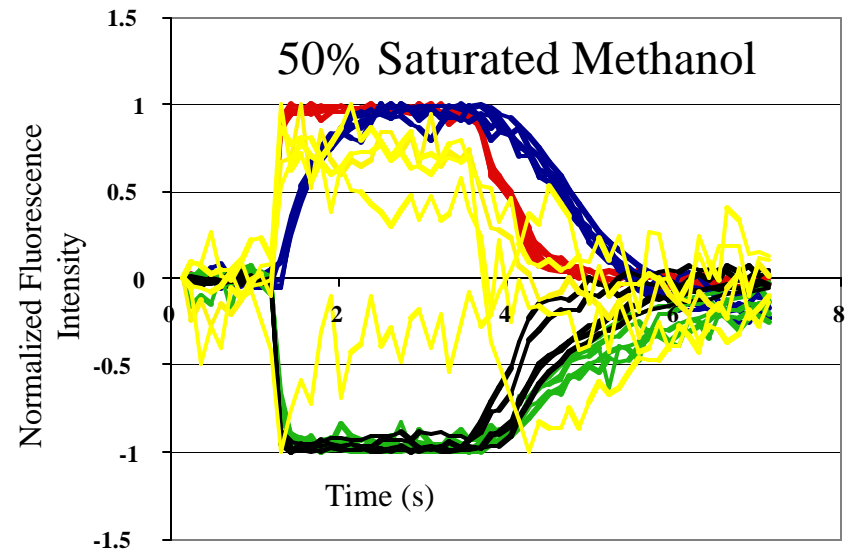
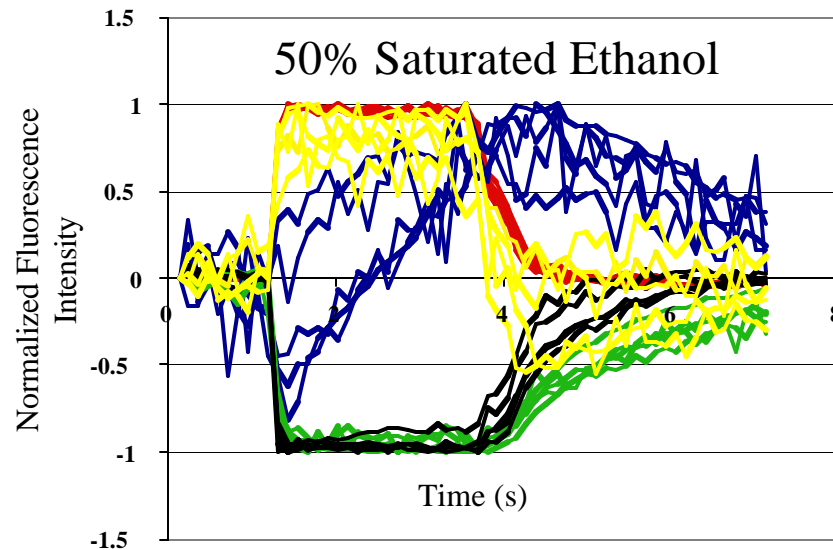
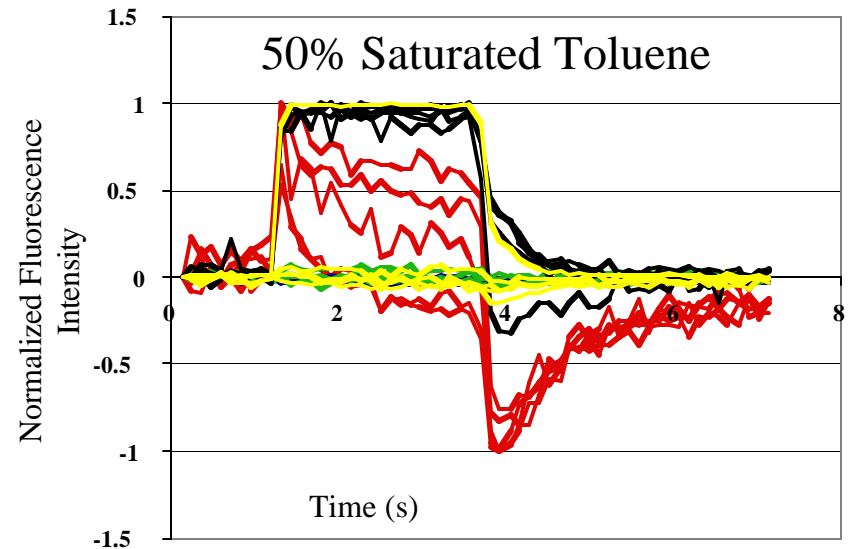
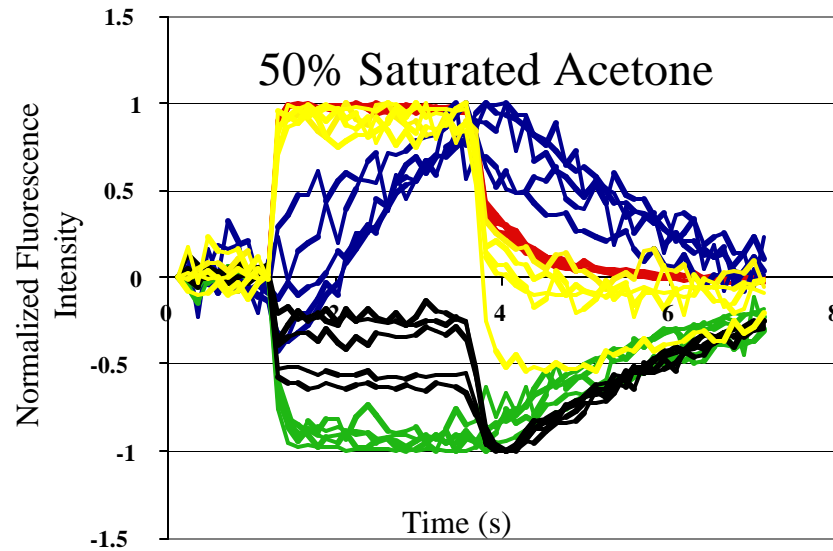
Randomly Ordered
5 Bead Array



Decoded 5
Bead Array



5 Sensor Types with 4 Analytes

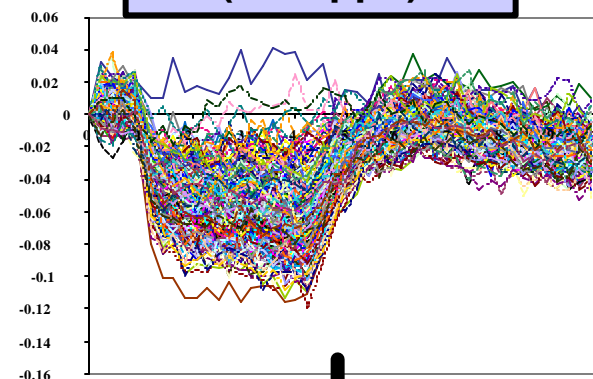
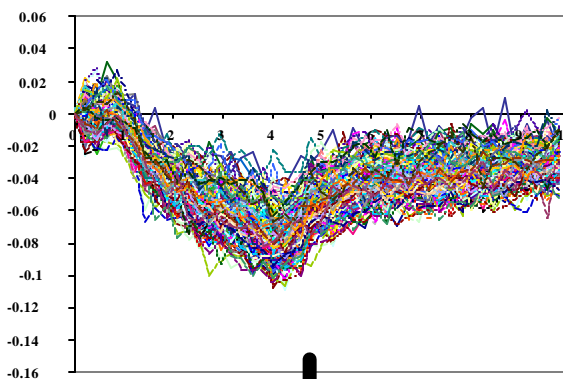
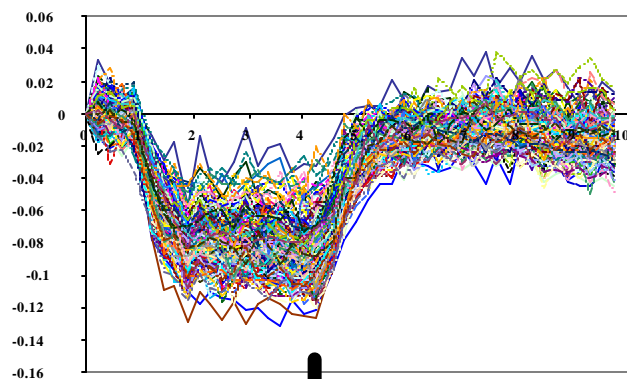


TIME (s) vs. FLUORESCENCE RESPONSE: 250 INDIVIDUAL Bead Sensors

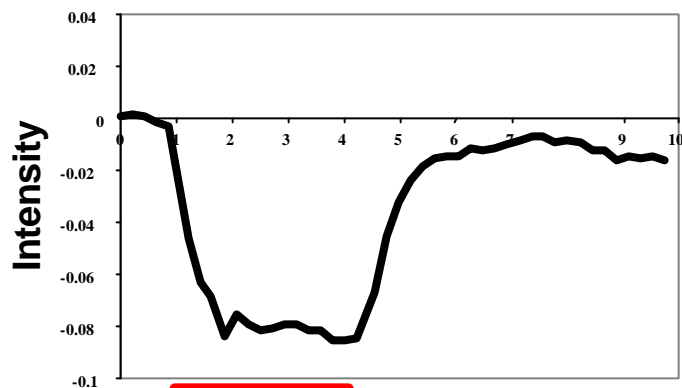
~23 ppb 2,4-DNT

~80 ppb 1,3-DNB

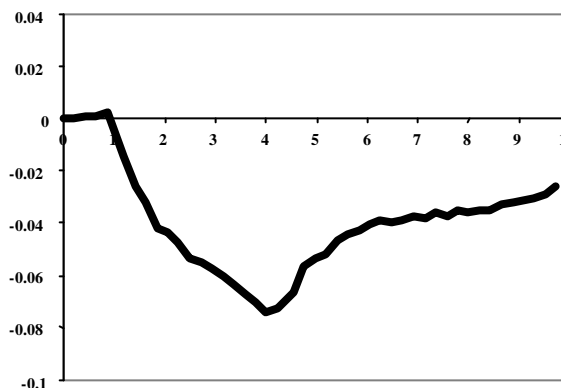
8% saturated
TNT vapor Strips
(~0.4 ppb)



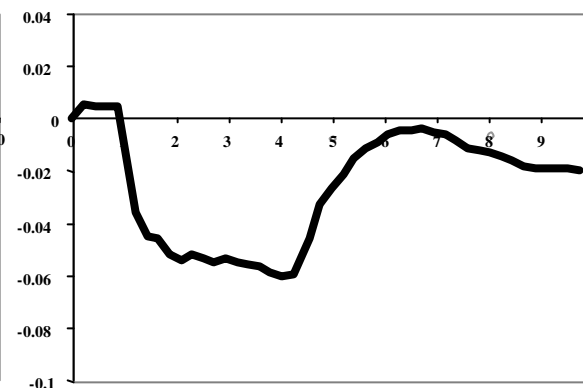
Signal/Noise Improvement:
Average of 1000 Sensors



3.25 s vapor exposure



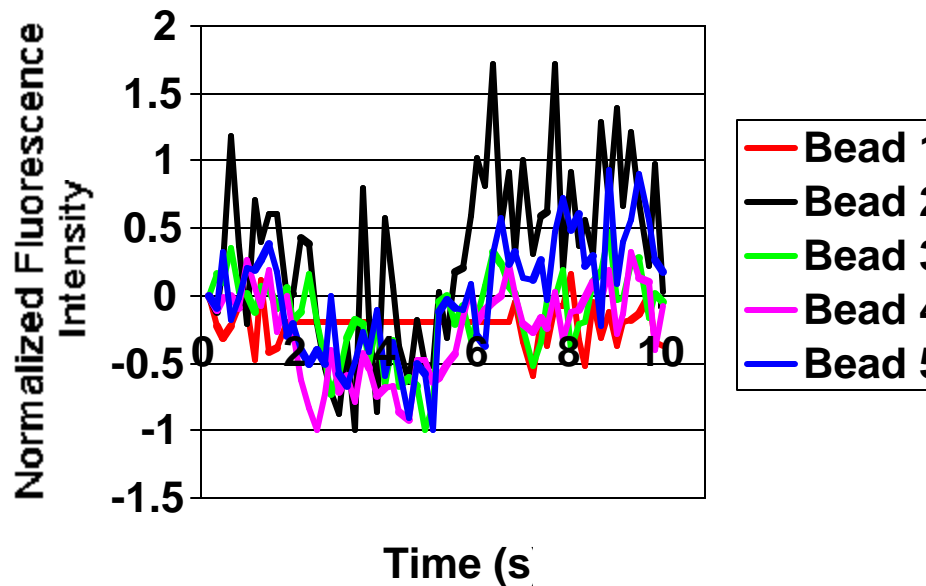
Time (s)



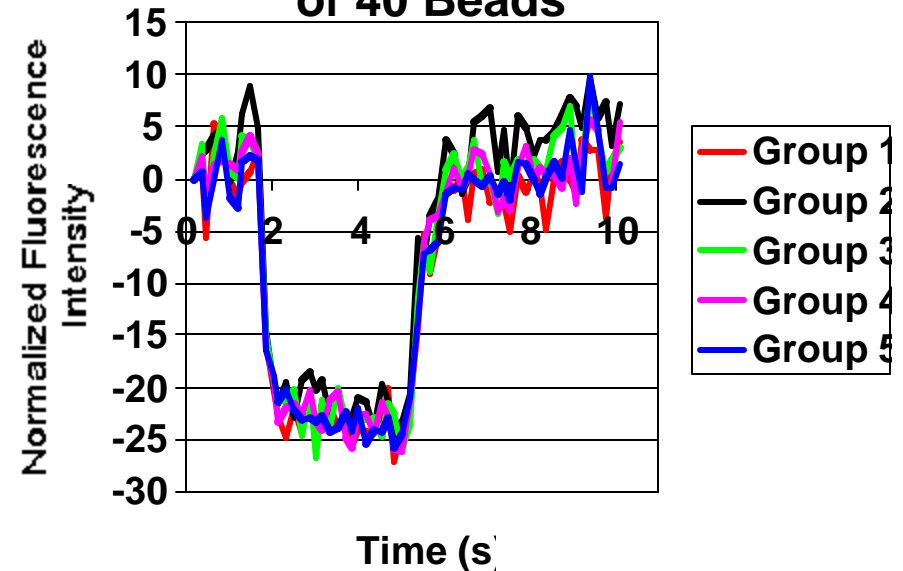
Albert, K. J. and D. R. Walt (2000) *Anal. Chem.* in press.

Signal Summing

**Individual Response of
5 Bead Sensors**



**Summed Responses of
5 Random Groups
of 40 Beads**



Summing improves signal-to-noise ratio.

Analytes for Two Class Problem

- Pure Analytes
 - Acetone
 - Benzene
 - Chloroform
 - Ethanol
 - Ethyl Acetate
 - Heptane
 - Methanol
 - Toluene
 - 1,3-Dinitrobenzene
 - 4-Nitrotoluene
- Binary Mixtures
 - Ethyl Acetate/Heptane
 - Methanol/Benzene
 - 4-NT/Benzene
 - 4-NT/Heptane
 - 4-NT/Methanol
 - 1,3-DNB/Ethyl Acetate
 - 1,3-DNB/Heptane

Concentrations of Analytes

Table 1: The concentration of the pure analytes $\pm 15\%$.

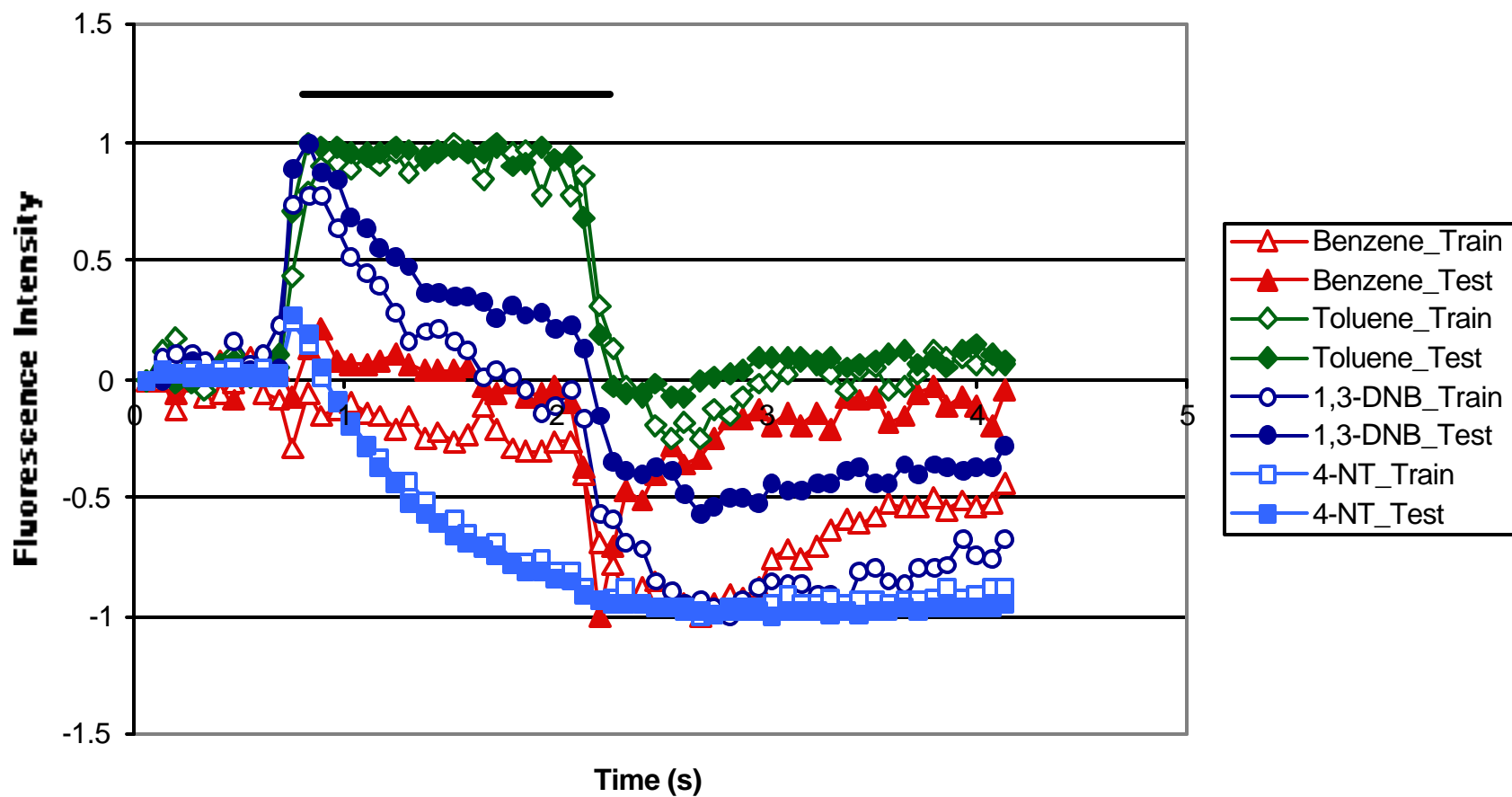
The concentrations were calculated based on literature values for analyte vapor pressures.

| Analyte | Vapor Pressure @25 °C (mmHg) | Concentration (ppm) |
|--------------------|---------------------------------|------------------------|
| Acetone | 2.31E+02 | 7.6E+04 |
| Benzene | 9.53E+01 | 3.1E+04 |
| Chloroform | 1.97E+02 | 6.5E+04 |
| Ethanol | 5.90E+01 | 1.9E+04 |
| Ethyl Acetate | 9.45E+01 | 3.1E+04 |
| Heptane | 4.57E+01 | 1.5E+04 |
| Methanol | 1.27E+02 | 4.2E+04 |
| Toluene | 2.84E+01 | 9.4E+03 |
| 1,3-Dinitrobenzene | 9.00E-04 | 6.0E-01 |
| 4-Nitrotoluene | 1.64E-01 | 1.1E+02 |

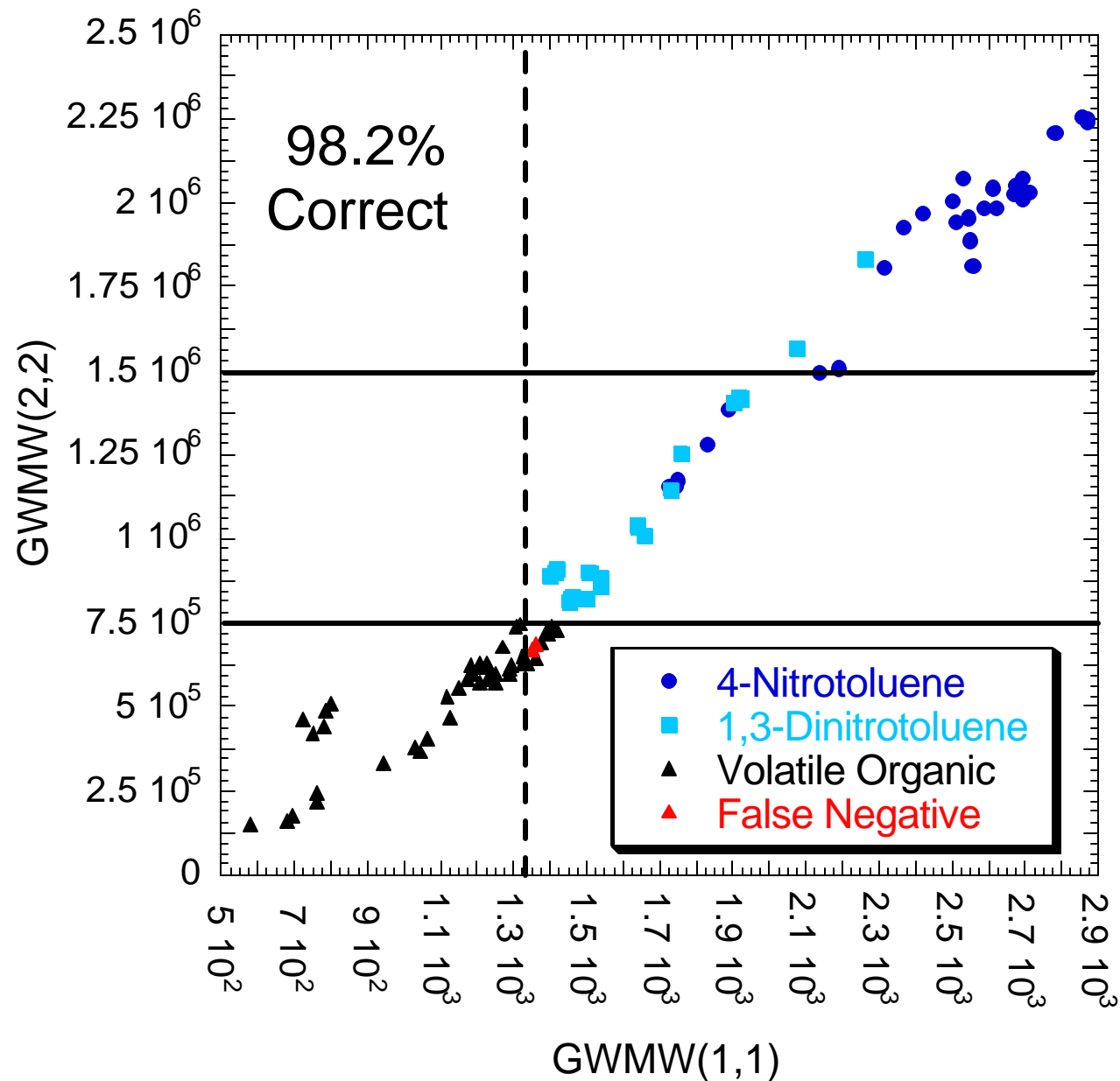
Table 2: The concentration of the binary mixtures $\pm 15\%$.

| Analyte 1 | Analyte 2 | Concentration analyte1 (ppm) | Concentration analyte2 (ppm) |
|---------------|--------------------|---------------------------------|---------------------------------|
| Benzene | Methanol | 3.1E+04 | 4.2E+04 |
| Benzene | 4-Nitrotoluene | 3.1E+04 | 5.5E+01 |
| Benzene | 4-Nitrotoluene | 3.1E+04 | 1.1E+02 |
| Ethyl Acetate | Heptane | 3.1E+04 | 1.5E+04 |
| Ethyl Acetate | 1,3-Dinitrotoluene | 3.1E+04 | 3.0E-01 |
| Ethyl Acetate | 1,3-Dinitrotoluene | 3.1E+04 | 6.0E-01 |
| Heptane | 1,3-Dinitrotoluene | 1.5E+04 | 6.0E-01 |
| Heptane | 4-Nitrotoluene | 1.5E+04 | 1.1E+02 |
| Methanol | 4-Nitrotoluene | 4.2E+04 | 5.5E+01 |
| Methanol | 4-Nitrotoluene | 4.2E+04 | 1.1E+02 |

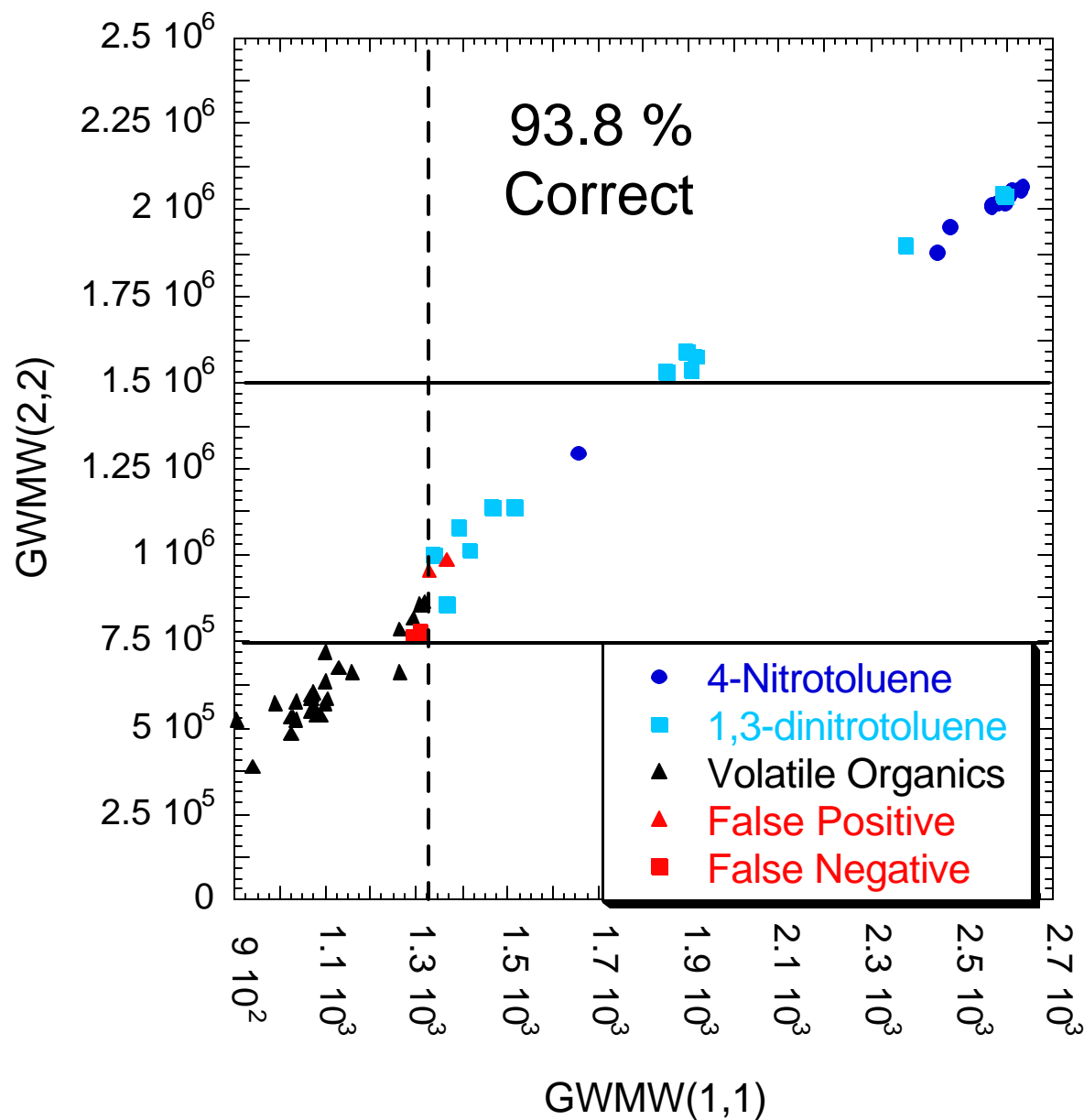
Reproducible Responses from Training to Testing array



First Testing Array (1 Month)



Second Test Array (7 months)



Live/Dead Bacteria Discrimination

Calculated Identity

| Actual Identity | | Live B10 | Live B4 | Live B5 | Live B8 | Live B9 | Dead B10 | Dead B4 | Dead B5 | Dead B8 | Dead B9 | Medium |
|-----------------|----------|----------|---------|---------|---------|---------|----------|---------|---------|---------|---------|--------|
| | Live B10 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Live B4 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Live B5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Live B8 | 0 | 0 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | Live B9 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Dead B10 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| | Dead B4 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| | Dead B5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| | Dead B8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| | Dead B9 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 |
| | Medium | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |

85% Correct, 87% Variance (7PCs)

B10: *Acintobacterium*

B4: *M. luteus*

B5: *E. coli*

B8: *Salmonella*

B9: *Klebsiella pneumoniae*

Acknowledgements

ONR



DARPA



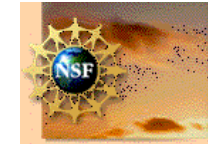
DOE



NIH



NSF



Walt Group

Myoyong Lee
Karri Michael
Paul Pantano
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Jane Ferguson
Michael Fleming
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Johns Hopkins University

Lenore Cowen

Illumina

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Kevin Gunderson

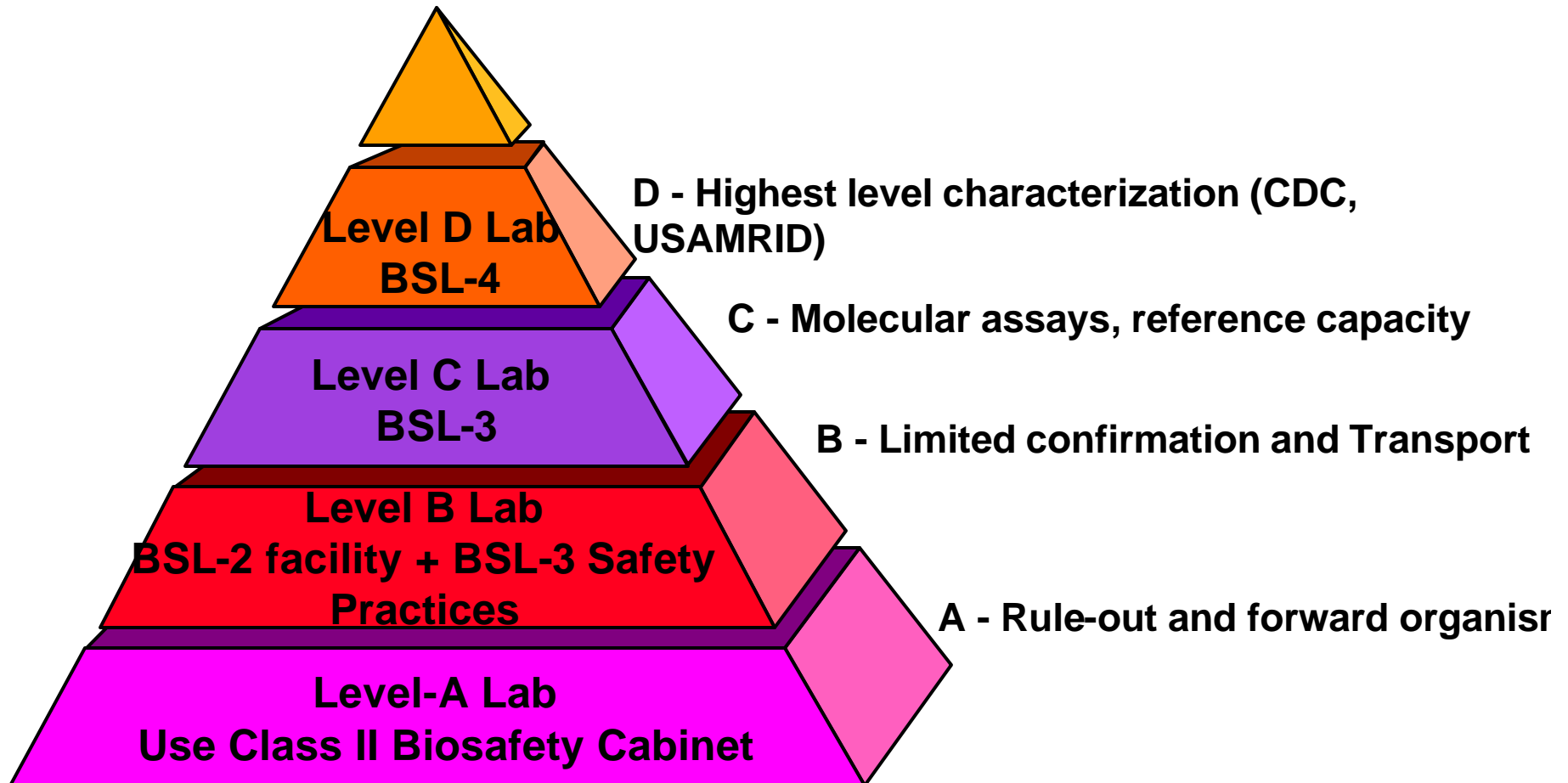
Laboratory Response Network

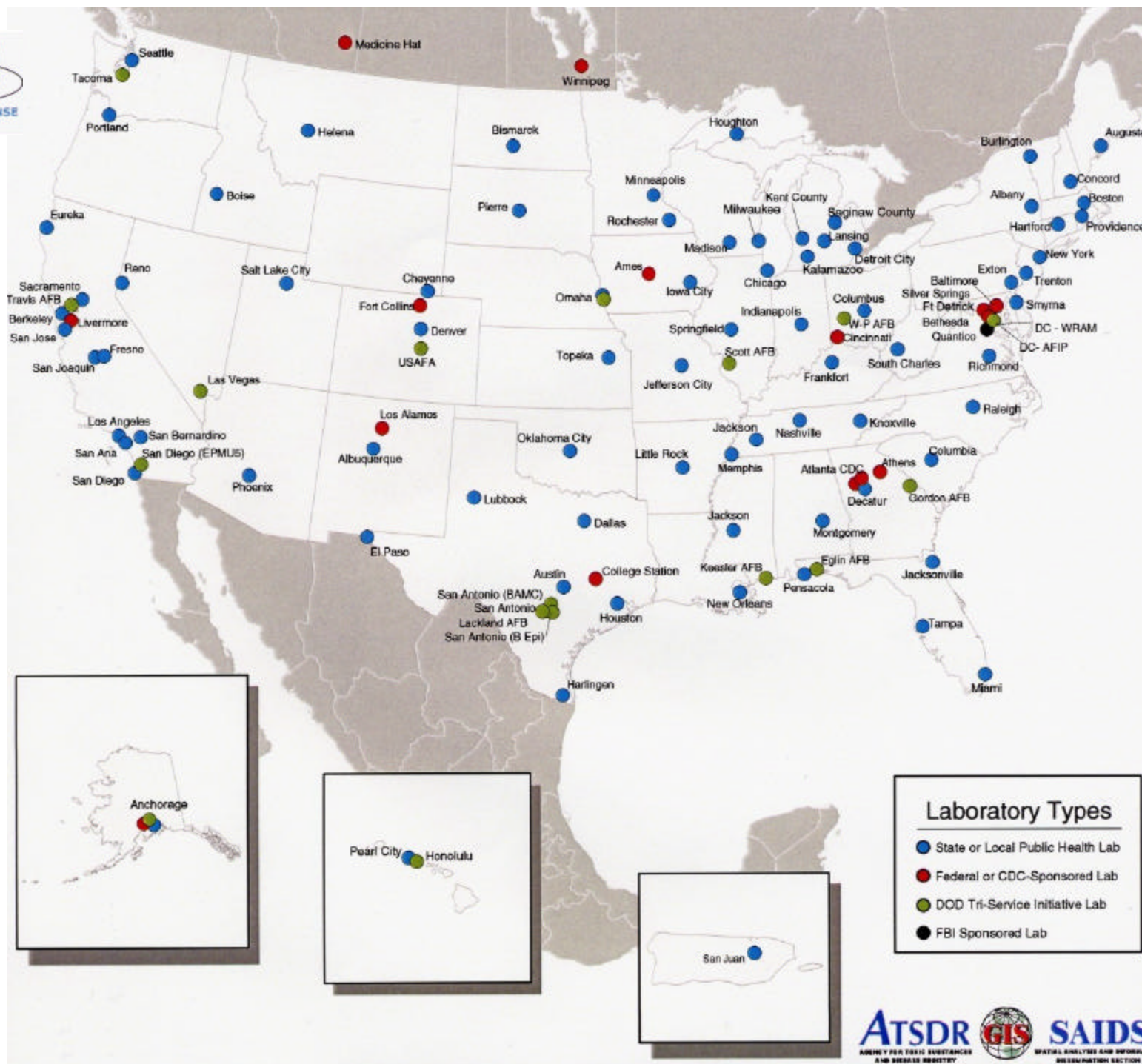
Ralph Timperi

Massachusetts Department of
Public Health, and

Association of Public Health
Laboratories (www.aphl.org)

Laboratory Response Network For Bioterrorism





CDC BT Rapid Response and Advanced Technology Lab

- **BSL -3**
- **Agent Identification and Specimen Triage**
- **Refer to and Assist Specialty Lab Confirmation**
- **Evaluate Rapid Detection Technology**
- **Rapid Response Team**

LRN Capacity

Specimen Collection and Transport

- **Appropriate specimens**
- **Forensic issues and chain of custody**
- **Timely transport & testing safety**

Capacity to Diagnose

- **Surveillance**
- **Rapid screens - People/environment**
- **Definitive and trusted testing**
- **Secure, reliable means of electronic communication**
- **The right answer, to the right persons at the right time**

LRN: Work-in-progress

- State and large city / county public health laboratories- secure internet website (reagents, protocols, capacity locator)
- Training and proficiency on 'highest priority agents'
- Conventional and rapid methods
- Validation of methods
- 'Surge capacity'

LRN: Growing capacity

- Clinical microbiology laboratories collaboration- standard protocols, rule-out testing for clinical specimens, (future) definitive identification of agents
- Building a secure system for electronic laboratory reporting of test results- the technology is not the problem
- Surge capacity- build, protect, access
- Technology and reagents to more laboratories- capacity to validate and accept

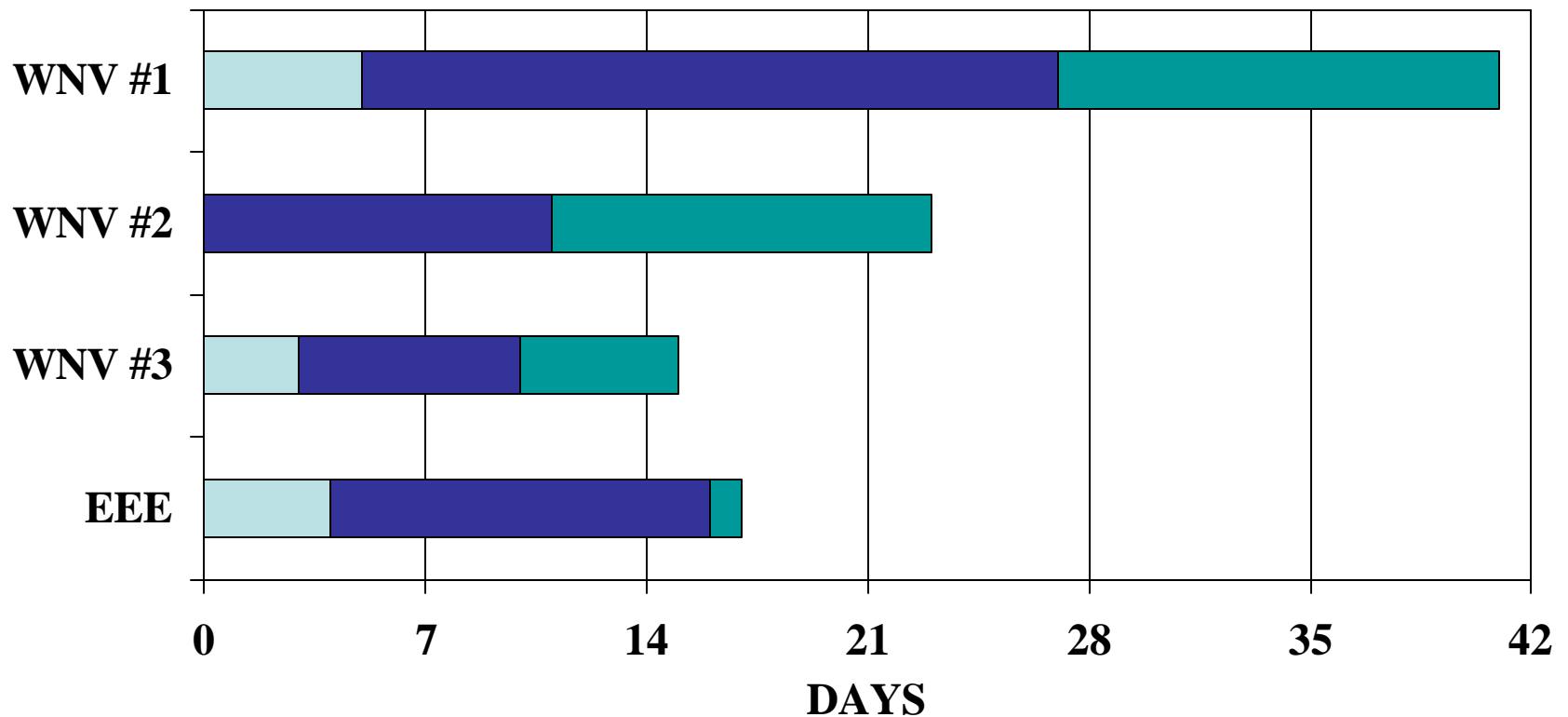
Laboratory and Testing Issues

- Surveillance- Numbers of ill persons, general syndromes, laboratory-based species and DNA characteristics
- Field testing- First responders, environmental, risk characterization
- Laboratory diagnosis of human and animal illnesses- coordination and communication
- 24/7 available and accessible capacity

Human Arbovirus Cases, MA

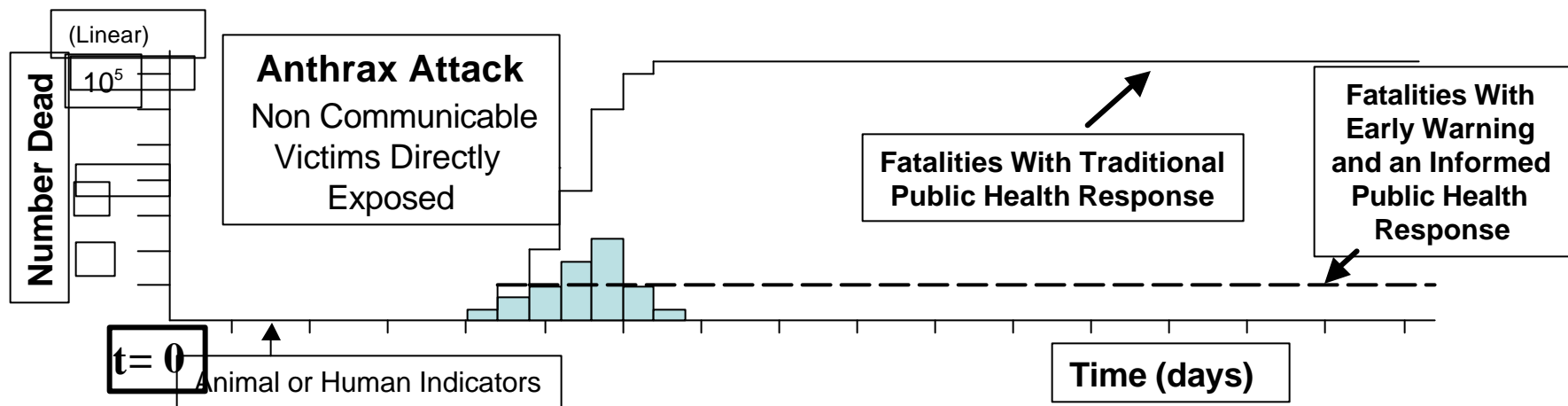
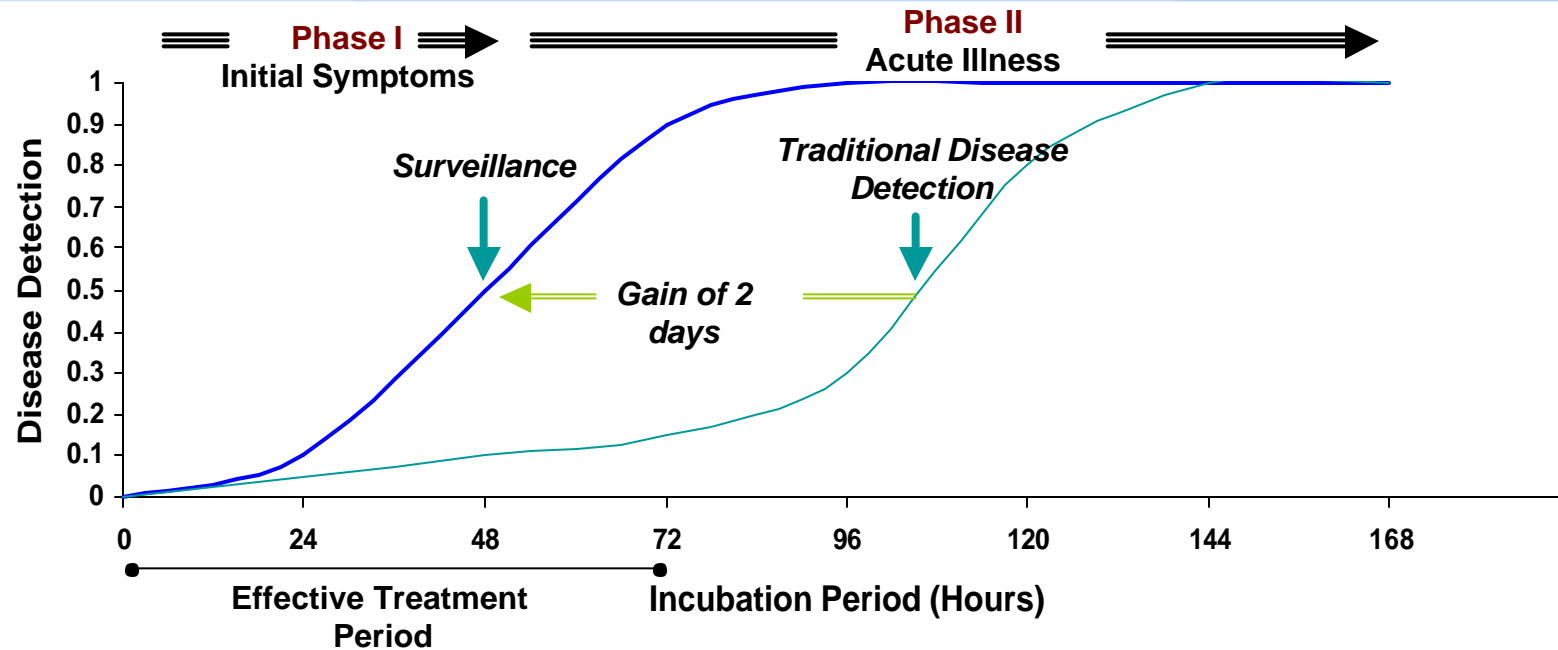
Timelines: Onset to Diagnosis

■ Onset to Health Care ■ Health Care to Notification ■ Notification to Diagnosis

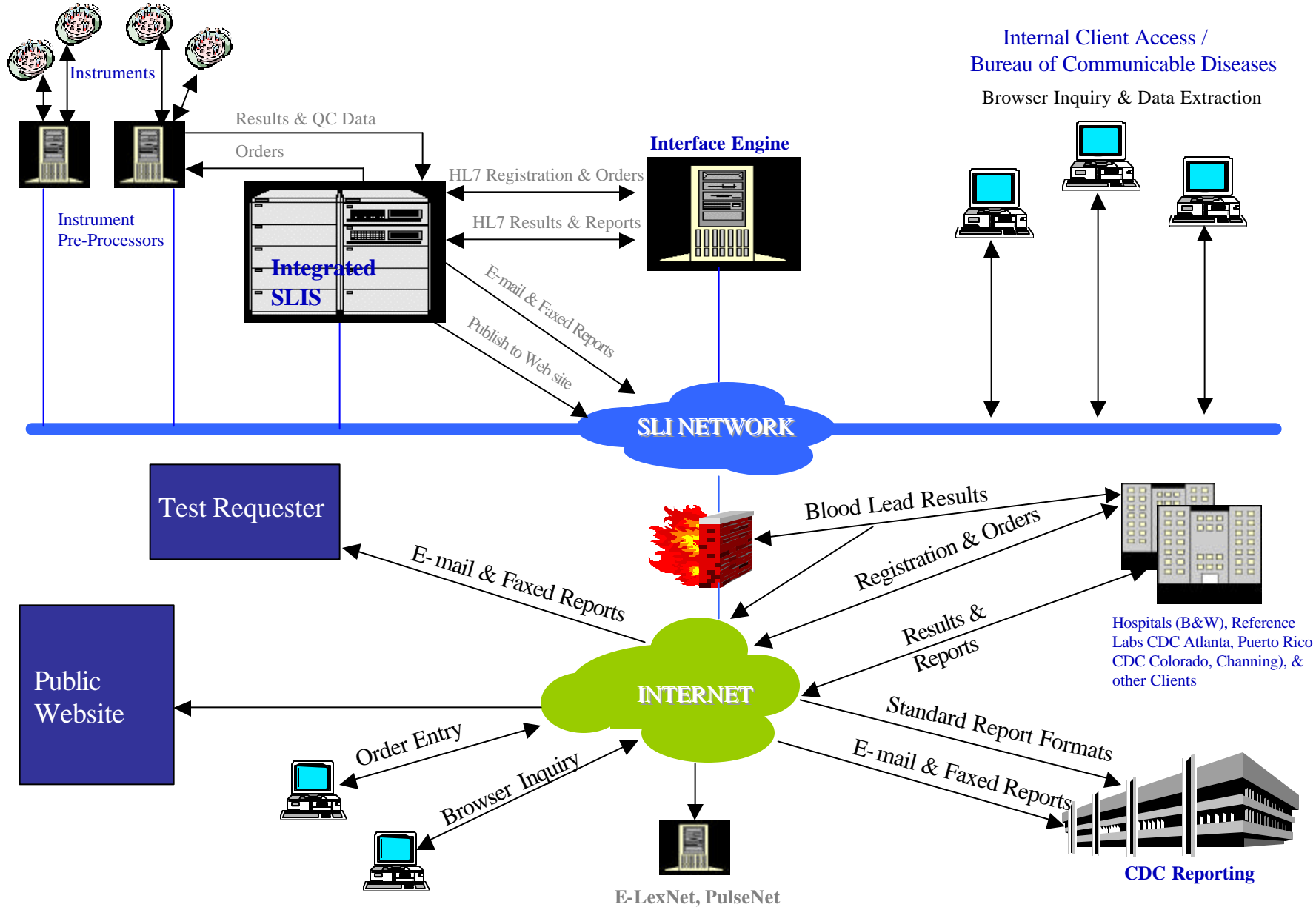




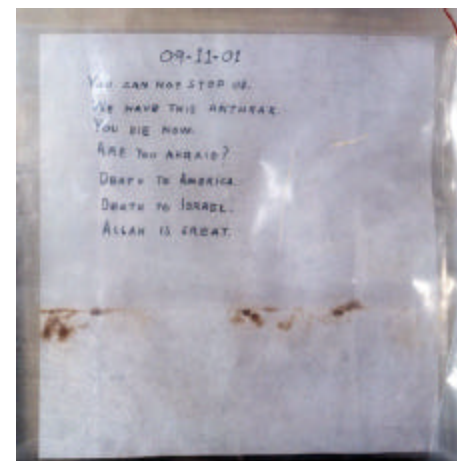
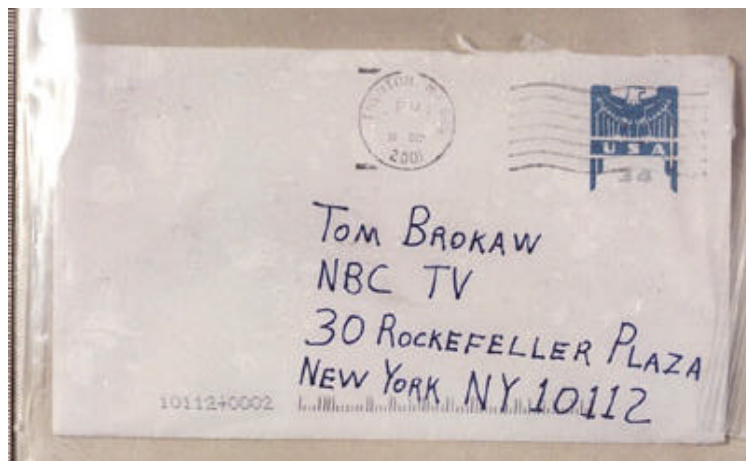
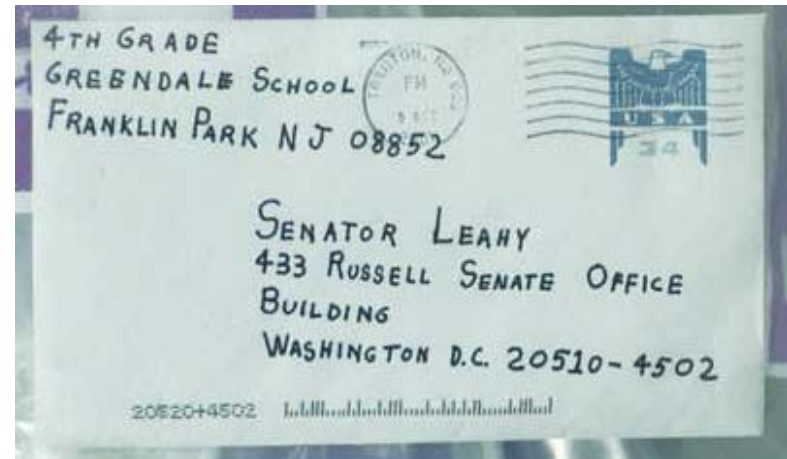
Impact of Surveillance on Survivability (Anthrax)



FRAMEWORK FOR AN INTEGRATED SLIS



Anthrax Sent By Mail September / October 2001



ENVIRONMENTAL SAMPLES

| Description | Number Submitted | Risk / Testing Priority |
|---|-------------------------|--------------------------------|
| U.S. Mail contaminated with anthrax | 0 | Low to high / High |
| U.S. Mail with a suspicious powder (not anthrax or other pathogen) with or without a threat letter | 52 | None / High |
| Powders, particulate matter and various liquid or solid material on surfaces of floors, walls, furniture, clothing, appliances or food | ~800 | None / Low |
| Clothing, household items, business products, etc. without evidence of powder, particulate matter, etc. | ~1800 | None / None |

LRN validated methods and reagents available

- ***Bacillus anthracis*: C, PCR, TRF**
***Brucella* sp.: C**
- ***Francisella tularensis*: C, TRF**
- ***Yersinia pestis*: C, PCR, TRF**
- ***Clostridium botulinum*: C**

**Conventional, polymerase chain reaction,
time resolved fluorescence**

Methods in development

- **Ricin: TRF**
- ***Brucella* sp.: PCR, TRF**
- ***Francisella tularensis*: PCR**
- ***Staph.* enterotoxin B: TRF**
- ***Burkholderia mallei*: PCR**
- ***Burkholderia pseudomallei*: PCR**
- ***Coxiella burnetii*: TRF**
- ***Clostridium botulinum*: EIA, TRF**
- **Validation in progress**
- **Validation by summer**
- **Validation by late summer**
- **EDA not estimated**
- **Fall/Winter 2002**
- **Fall/Winter 2002**
- **EDA not estimated**
- **2004**

Testing Methods - Environmental

- 1- Gross examination- (environmental samples only)
- 2- Microscopic examination for bacteria and spores
- 3- DNA test methods
- 4- Culture (growth of bacteria on artificial media)
- Most samples tested by methods 1, 4
- U.S. Mail and similar items tested by methods 1, 2, 4 and possibly 3
- Some items with no apparent contamination, no risk indicators tested by method 1 only

Targeting Immunity to Biothreats

David Scadden

Massachusetts General Hospital

Harvard Medical School

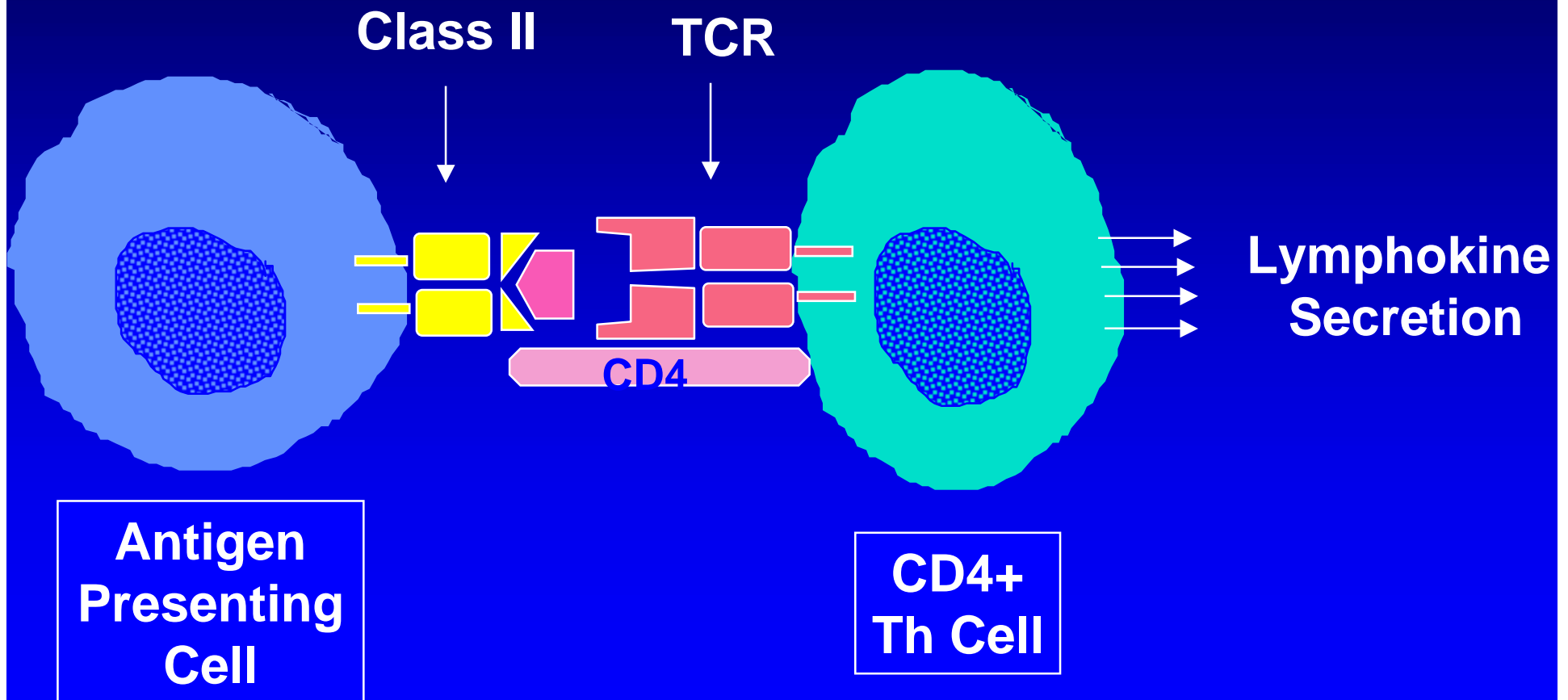
Cellular immunity and HIV disease

Immune control of HIV infection is possible without anti-retroviral therapy

Evidence for CTL control of HIV

- Negative correlation between CTL and viral load by more sensitive assays (Ogg et al)
- Increase in SIV viremia with CD8 cell depletion (Schmitz et al; Jin et al)
- Association between appearance of CTL and decline in viremia in acute infection (Koup et al; Borrow et al)

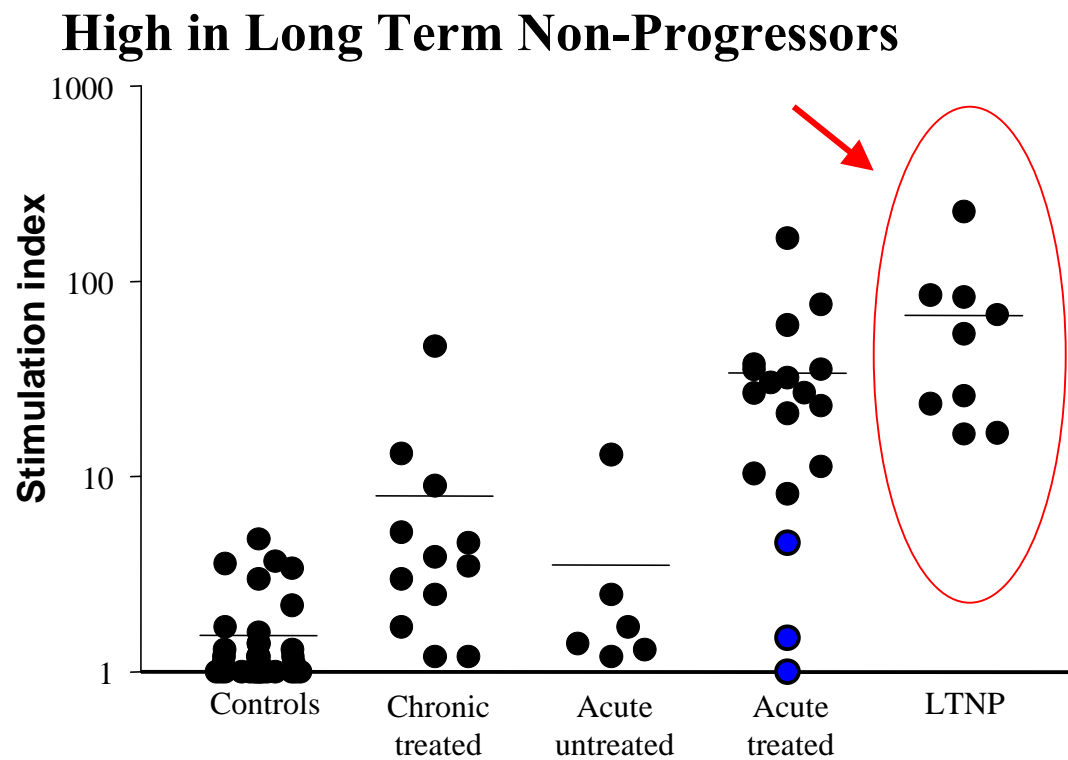
Optimal CTL function depends on virus-specific T helper cells



**HIV-specific CD4+ T cell
responses are associated with
control of HIV**

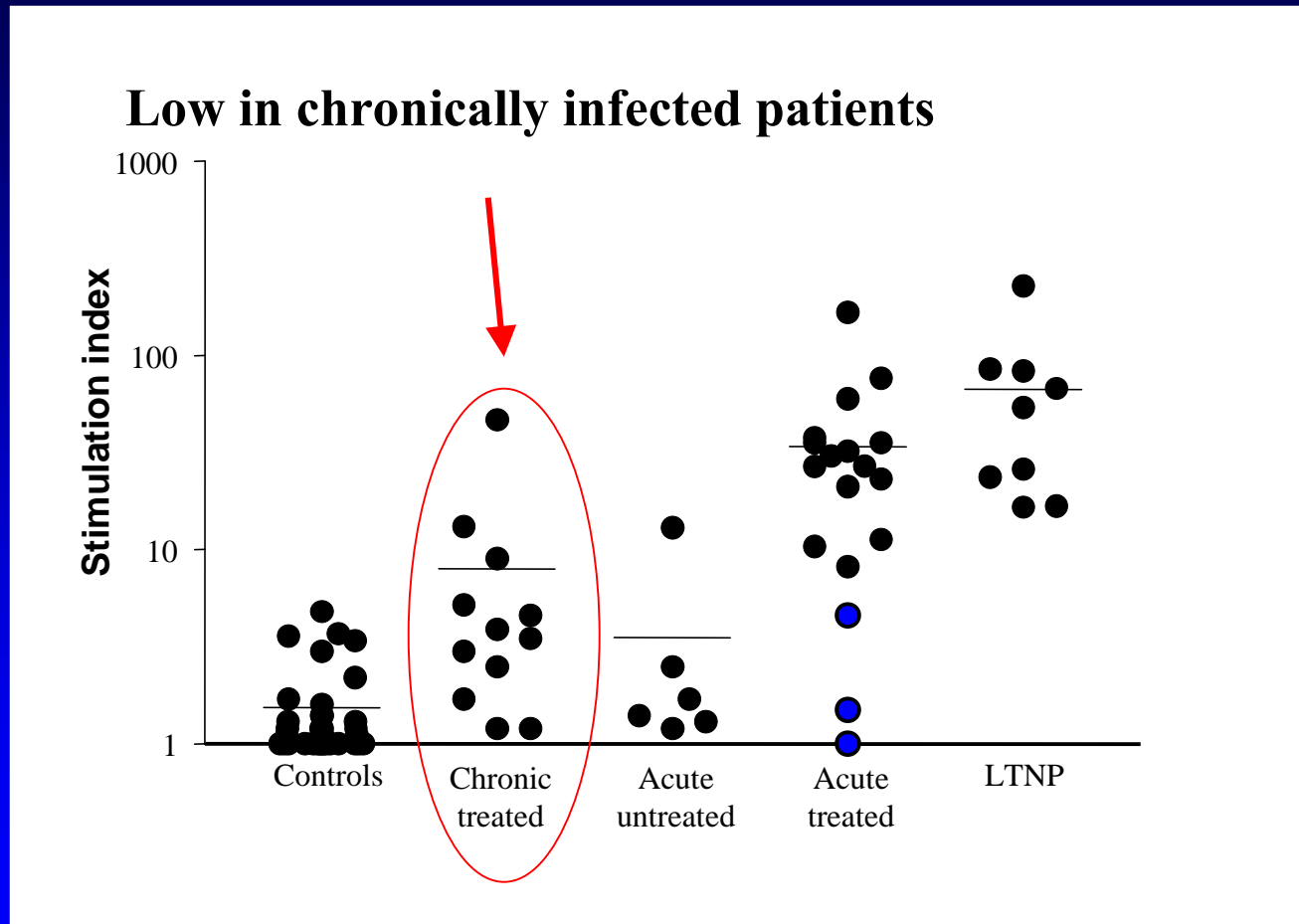
Rosenberg et al. Science 1997; 278, 1447

HIV specific helper T cell function



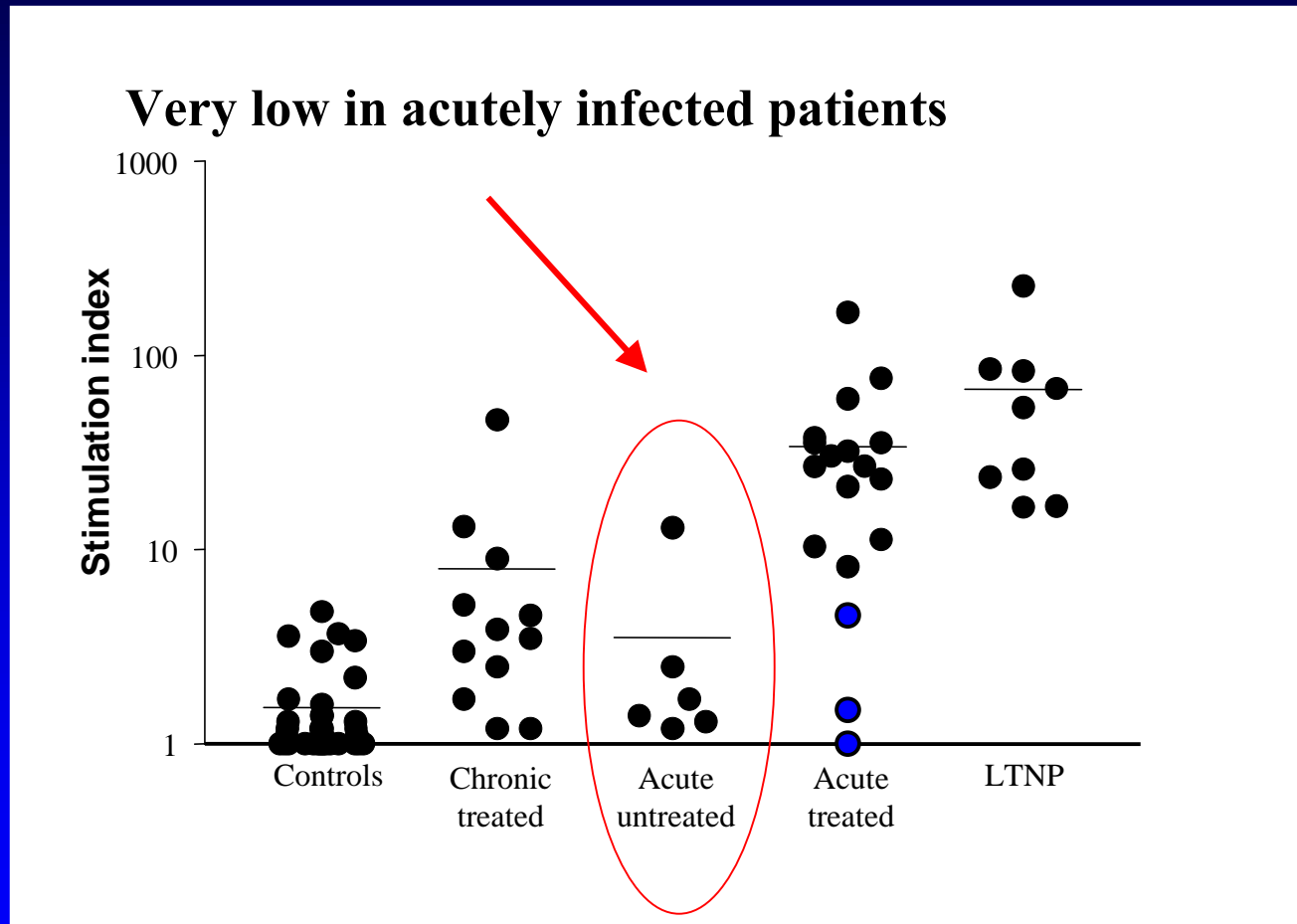
Rosenberg et al. Nature 2000; 407, 523

HIV specific helper T cell function



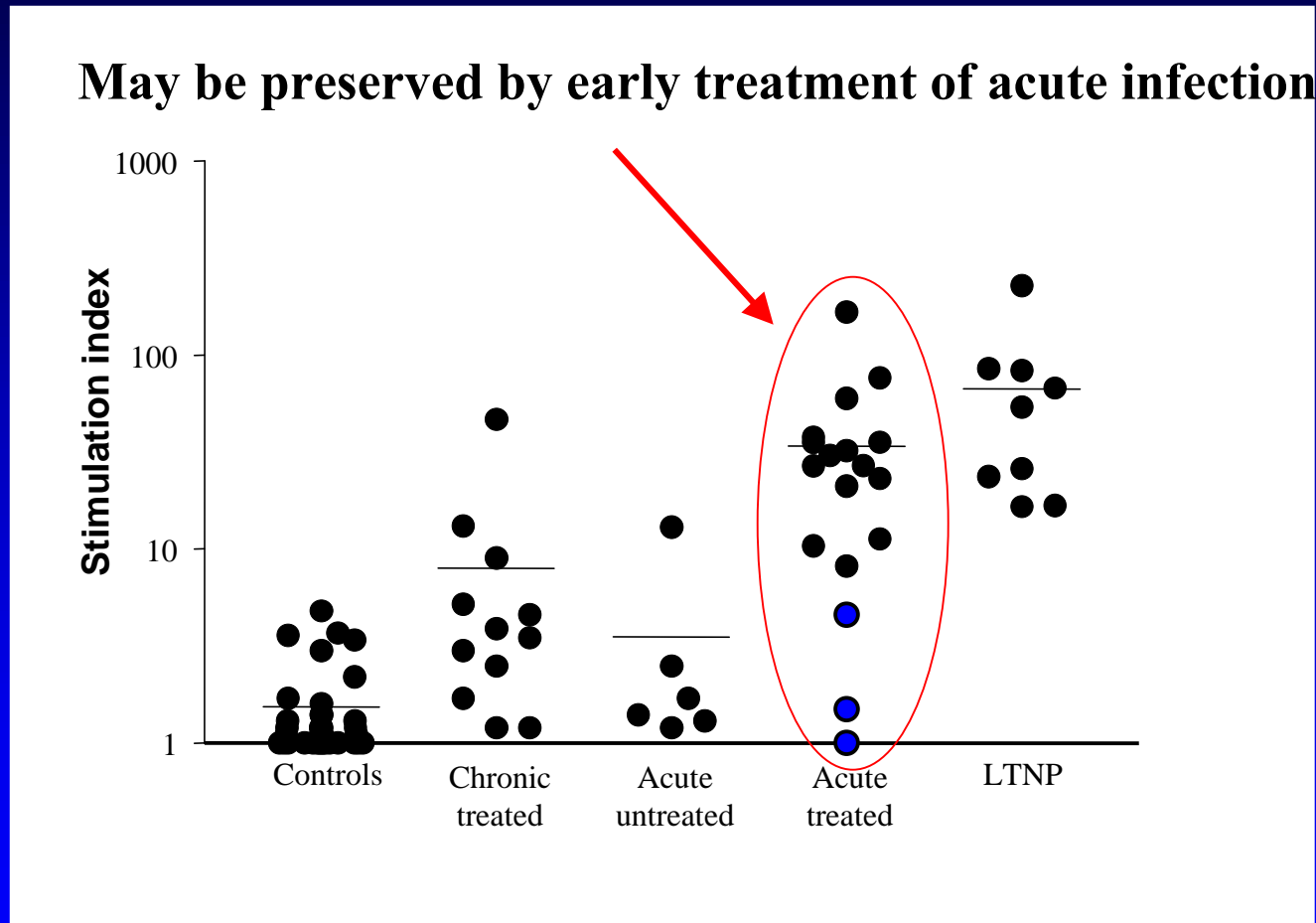
Rosenberg et al. Nature 2000; 407, 523

HIV specific helper T cell function



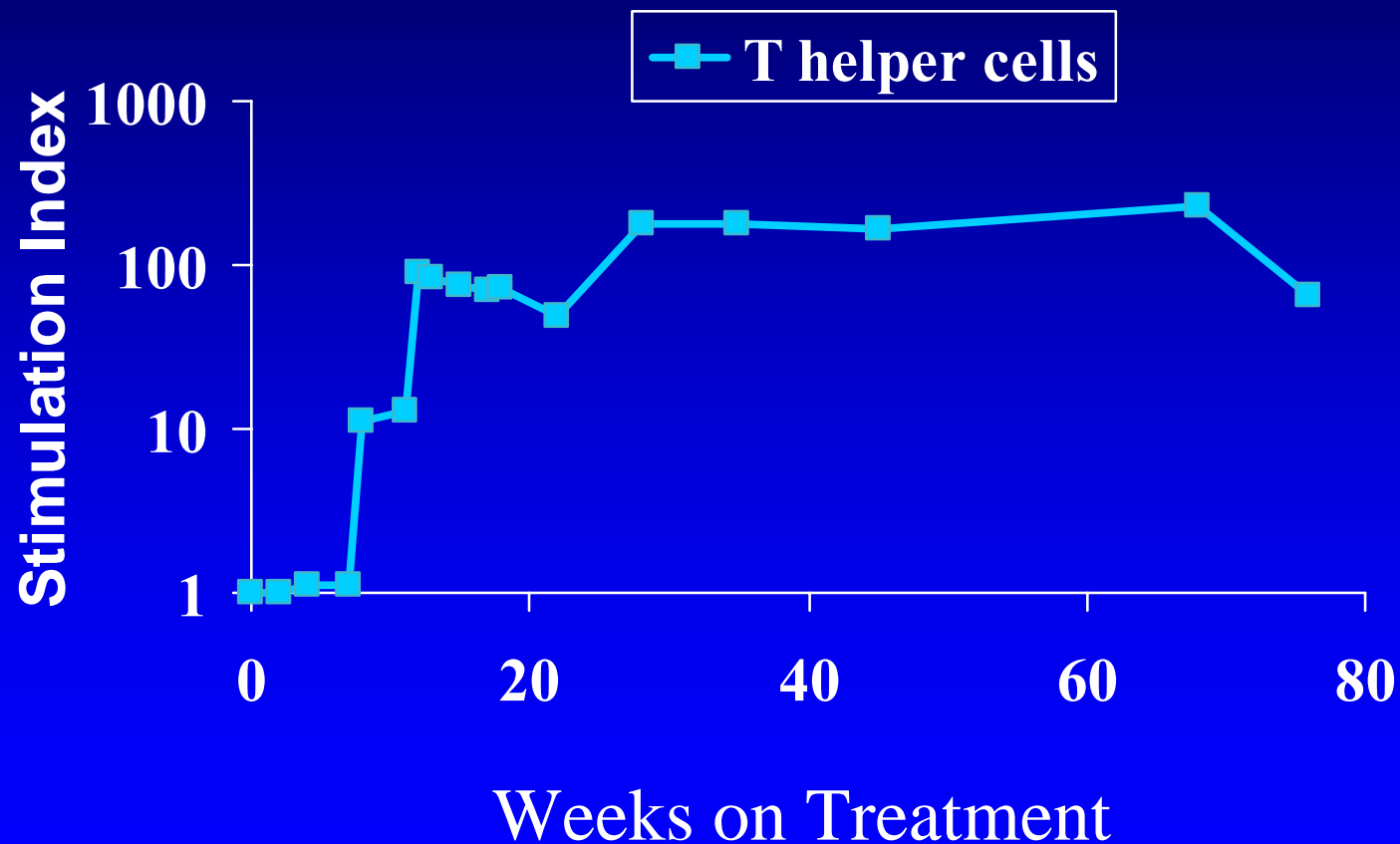
Rosenberg et al. Nature 2000; 407, 523

HIV specific helper T cell function

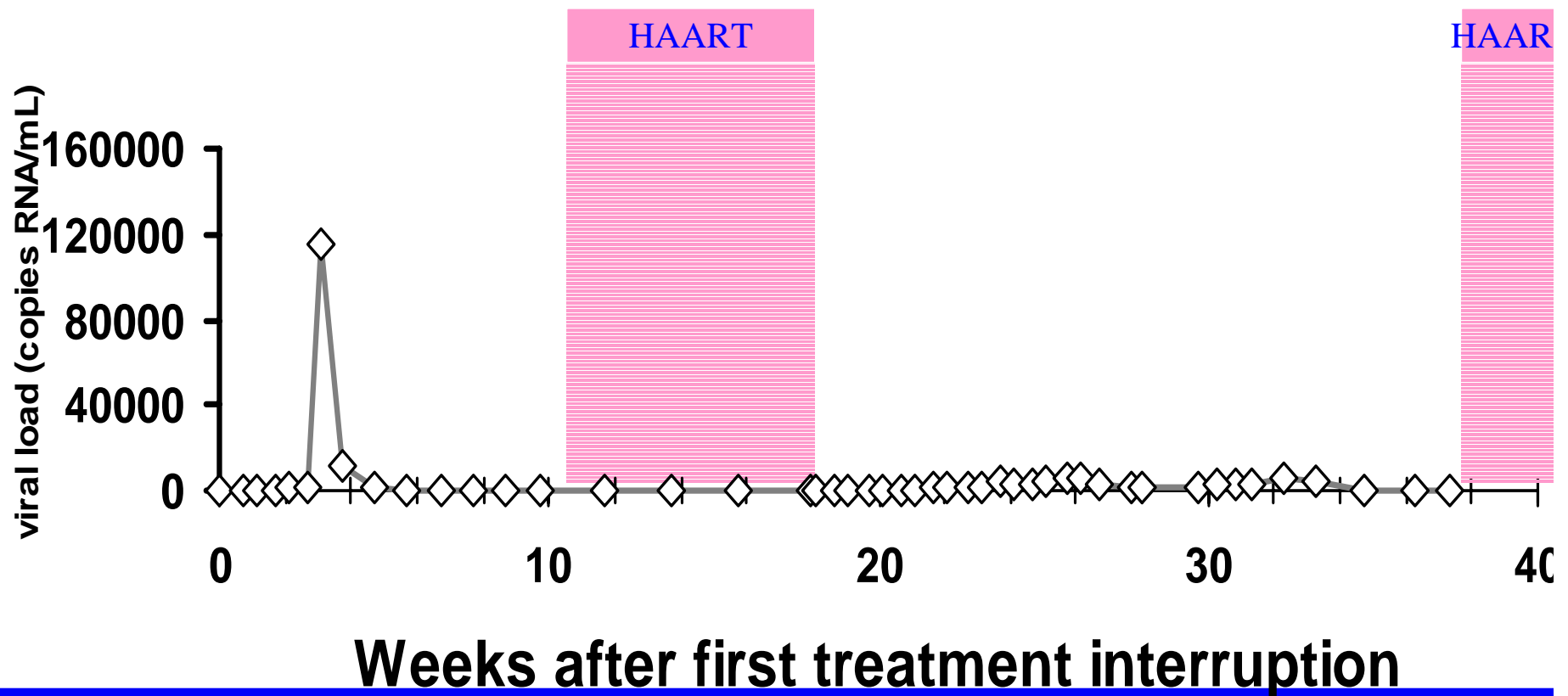


Rosenberg et al. Nature 2000; 407, 523

Treatment of acute HIV-1 infection results in augmentation of T helper cell responses



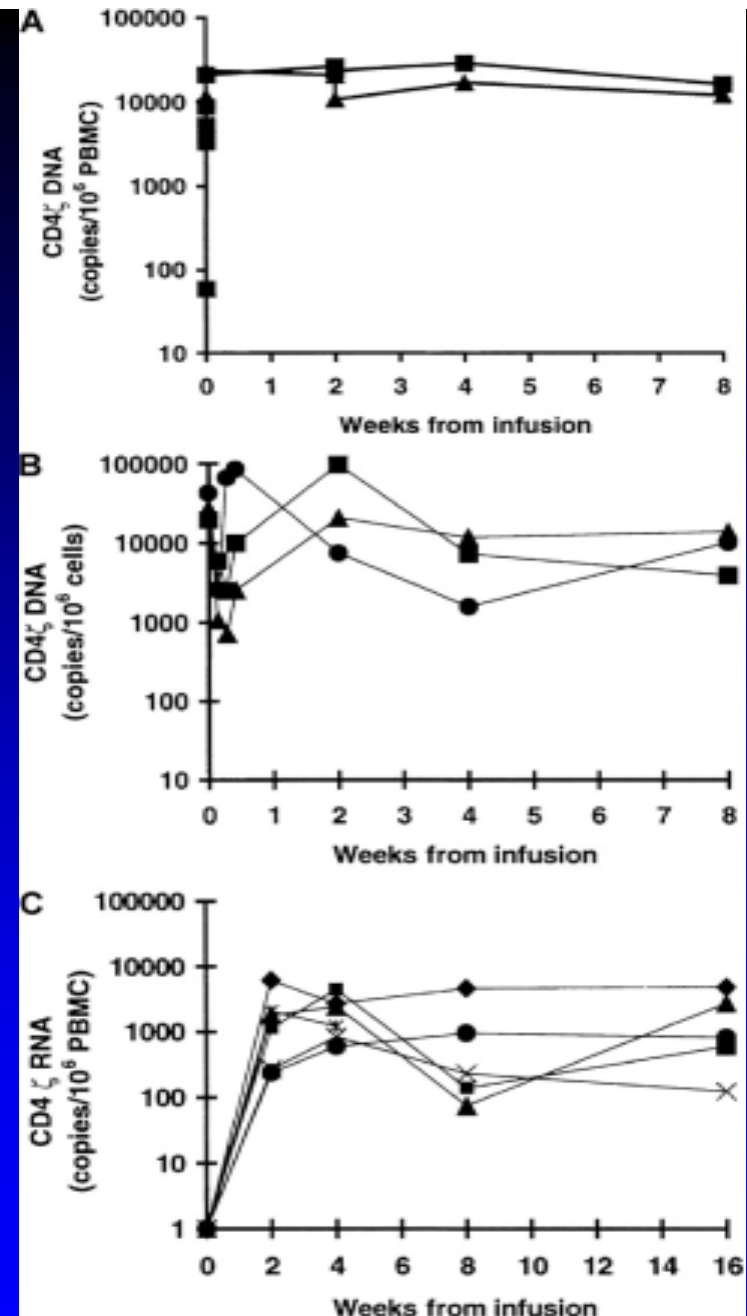
Preserved HIV specific T cell helper function is associated with control of HIV without HAART



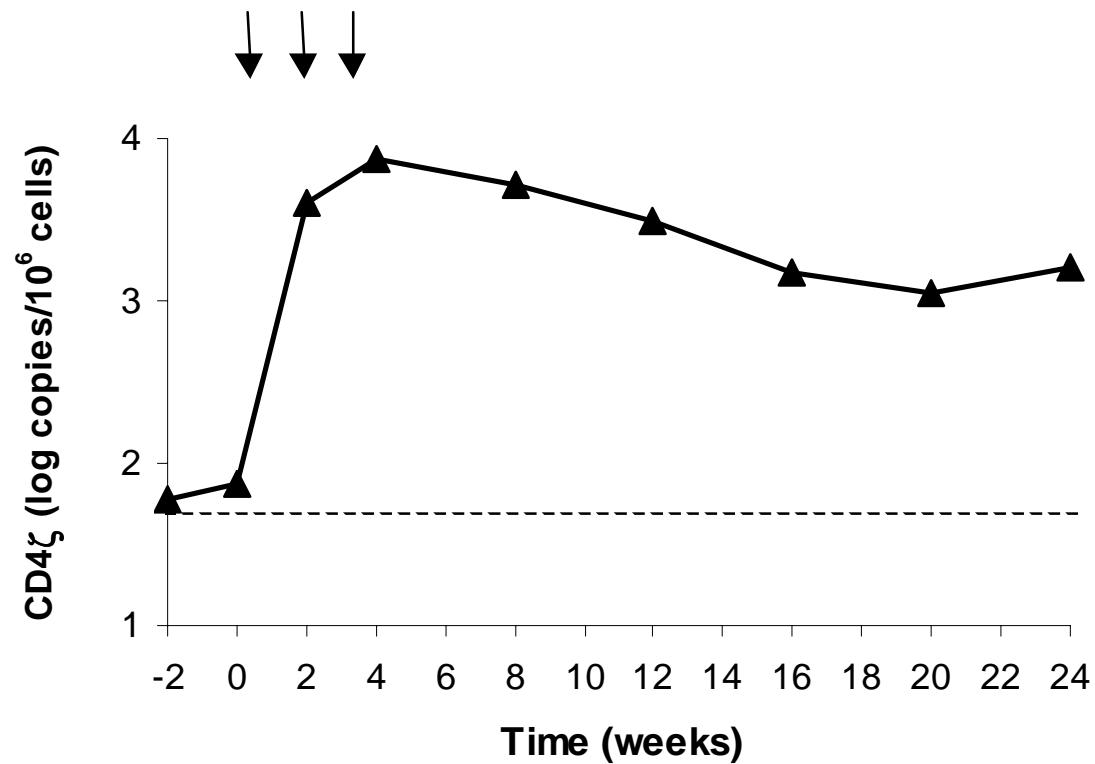
**Structured treatment
interruptions (STI) in acute
HIV infection may result in
immunologic control of viremia**

CD4 ζ -modified T-cell survival and gene expression in peripheral blood mononuclear cells (PBMCs)

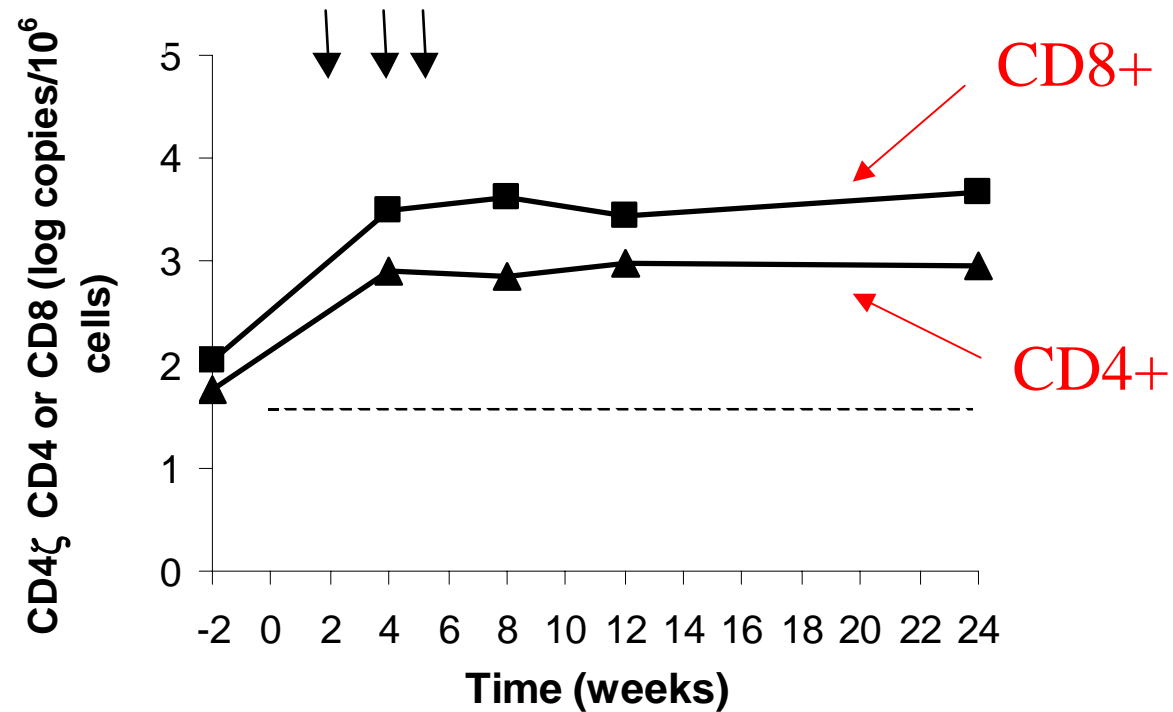
Mitsuyasu et al, *Blood* 2000; 96:785



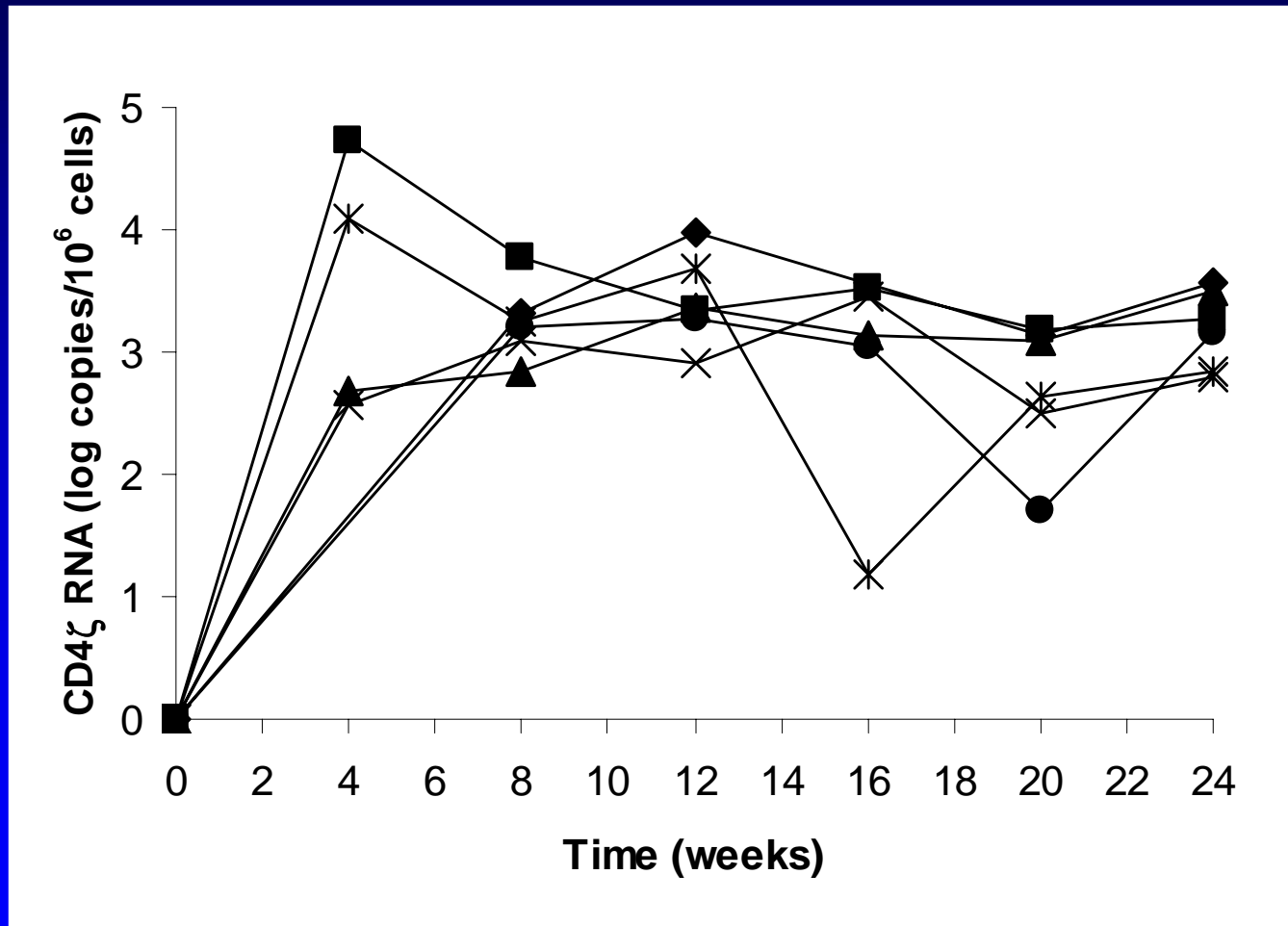
Persistence of cells with chimeric TCR DNA



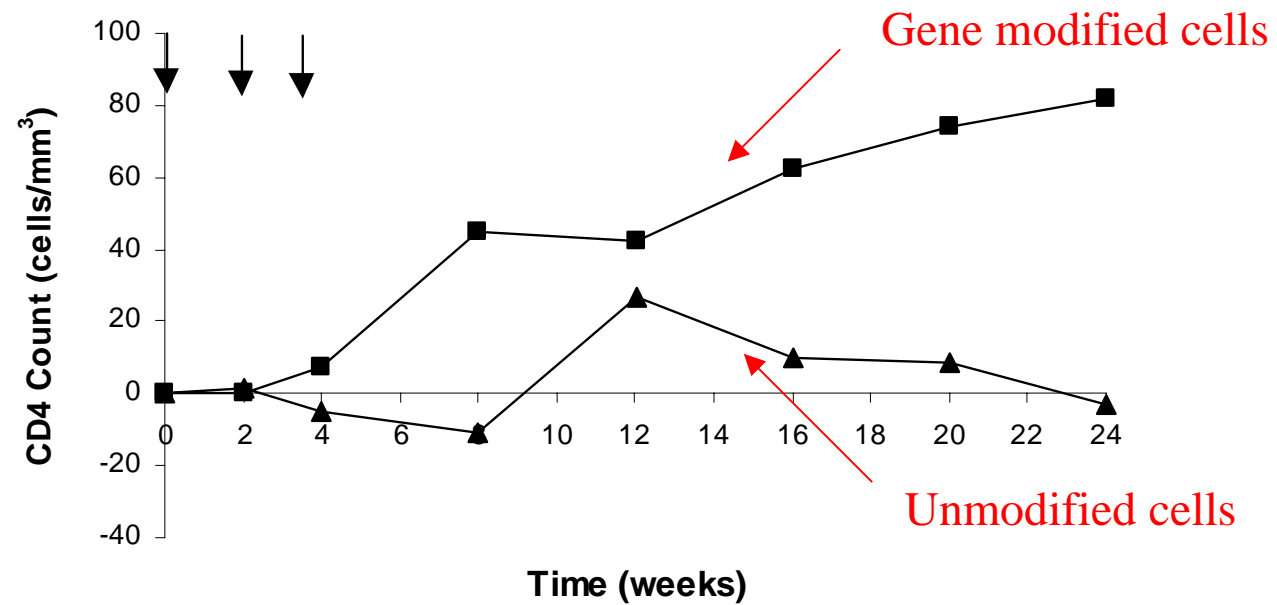
Persistence of cells with chimeric TCR DNA



Persistence of cells expressing chimeric TCR RNA



CD4+ T cell counts after cell infusions



Plasma viral load over time

